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DETERMINANTS OF ORGAN TROPISM OF SENDAI VIRUS

M. Tashiro [1], N.L. McQueen [2], and J.T. Seto [2]

[1] Department of Viral Diseases and Vaccine Control, National Institute of Infectious Diseases, Tokyo, 208-0011 Japan,
[2] Department of Microbiology, California State University, Los Angeles, Los Angeles, California, 90032-8201

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1. ABSTRACT

Wild-type Sendai virus is exclusively pneumotropic in mice. Protease activation mutants, ts-f1 and F1-R, were isolated from persistently infected tissue culture cells. Additional mutants were isolated from wild-type Sendai virus with phenotypes similar to the pantropic mutant, F1-R. The genome of the mutants was sequenced and mutations were revealed in several proteins encoded by the genes. Three of the six mutations in the fusion (F) proteins were considered prime candidates for the determinant of pantropism. Characterization of the mutants led to the finding that the exchange (Ser to Pro) residue 115 next to the cleavage site of the F protein was the primary determinant that resulted in the enhanced cleavability of the F protein. Another important finding was bipolar budding of F1-R in polarized epithelial cells and mouse bronchial epithelium. This has been attributed to two mutations in the matrix (M) protein, at residues 128 (Asp to Gly) and 210 (Ile to Thr). Thus the determinants of pantropism of F1-R are protease activation of the F protein and bipolar budding attributed to the mutated M protein and enhanced disruption of microtubules.

2. INTRODUCTION

The focus of our investigations has been on the molecular biology of the pathogenesis of paramyxoviruses, more specifically on the role of proteolytic cleavage of the viral glycoproteins. Host and organ tropism, spread of infection in the organism, and pathogenesis of paramyxoviruses have been well documented to be correlated with cleavage activation of the viral glycoproteins, and the host protease dependent expression of viral pathogenicity (1,2).

Sendai virus, the prototype of paramyxovirus, is a counterpart of human parainfluenza virus type 1 (HPIV1), which causes a respiratory infection in children. Sendai virus, isolated during an investigation of an outbreak of pneumonitis of the newborn (3), is exclusively pneumotropic in mice and readily establishes persistent infections in tissue cultures. Sendai virus has been used as a model for investigations on the *in vivo* mechanism of protease-mediated infections of respiratory agents (2).

Sendai virus has two glycoproteins, HN and F proteins. HN is for attachment of virus to cellular receptors and F is for cell fusion. Post-translational proteolytic cleavage of Fo into F1 and F2 subunits is essential for cell fusion, penetration, and activation of infectivity (4,5). The cleavage site of F protein consists of a single arginine residue. It is cleavable *in vitro* by trypsin. In embryonated eggs, Sendai virus is readily propagated in the infectious form by the proteins activating protease shown to be a blood clotting factor Xa, a vitamin K-dependent serine protease of the prothrombin family. It is secreted by the lining of the epithelial membrane cells into the allantoic and amniotic cavities (6,7). In the respiratory organs of rodents, progeny viruses are produced in the activated form and multiple cycles of replication take place resulting in extensive pathological damage in the lung. Tryptase Clara, a tryspin-like serine protease, has been identified in Clara cells, a secretory non-ciliated cell present in the bronchial and bronchiolar epithelium. Tryptase Clara has been shown to be a host protease that cleaves behind the single arginine residue at the site of cleavage of the F glycoprotein (8,9).

We proposed, that during long term persistent infections by paramyxoviruses, crisis or the fatal form of the disease may occur owing to the selection pressures that result in the preferential growth of protease activation mutants (10). Such a temperature sensitive (ts) host range mutant, ts-f1, has been isolated from persistently infected MDCK cells (11). Another host range mutant was isolated from ts-f1; it has been designated F1-R and found to be pantropic or cause a systemic infection in mice (12).

3. SENDAI VIRUS

3.1. Genome and proteins

The genome of Sendai virus, a negative strand RNA virus, consists of 15,384 nucleotides with the gene order of 3'NP-P/C/V-M-F-HN-L-5' (13,14,15). The genome codes for the nucleocapsid (NP), phosphoprotein (P), matrix (M), fusion (F), hemagglutinin-neuraminidase (HN), and large (L) proteins, respectively. The entire genome of Sendai virus (Z strain), of the two host range mutants (ts-f1 and F1-R), and a revertant (T-5) derived from the pantropic mutant (F1-R) has been sequenced (13,14,15,16).

3.2. Molecular biology

In search of the molecular basis of the virulence of paramyxoviruses, the focus has been on the role of the F glycoprotein. In infected tissue cultures multiple cycles of viral replication are the consequence of proteolytic cleavage of the F protein (1). Activation of the viral infectivity is dependent upon post-translational cleavage of the glycoprotein precursor Fo to F 1 and F 2 (4,5). For wild-type Sendai virus cleavage in vivo is only by tryptase Clara, a trypsin-like host protease secreted by Clara cells that are restricted to the respiratory epithelium (8,9). Arginine is the residue at the cleavage site of wild-type virus F protein. The F protein of the pantropic mutant, F1-R, has a proline residue at the cleavage site (17) which renders the protein cleavable by ubiquitous host protease(s). Therefore, cleavage activation and mutiple cycles of replication of the wild-type virus occur exclusively in the lungs, whereas, for F1-R, they occur in many organs. Thus, the primary determinant of pantropism is proteolytic cleavability and the organ distribution of the proteases (12,18,19).

3.3. Budding domain

In addition to proteolytic cleavage of the F glycoprotein, the budding polarity of F1-R has been proposed to be a determinant of pantropism. Another important difference between F1-R and wild-type Sendai virus has been identified. The mutant was shown to bud bipolarly, at the apical and basolateral domains of the plasma membrane, in the bronchial epithelial cells of mice and in polarized MDCK cells, whereas budding of wild-type virus was at the apical domain (18,19). Specific viruses have been shown to be targeted to the apical domain and other viruses to the basolateral domain (20). For example, paramyxoviruses and influenza viruses bud at the apical domain whereas HIV and vesicular stomatitis viruses bud at the basolateral surfaces. The latter viruses are systemic agents. Systemic mutants of influenza virus bud at the apical domain. It has been postulated that budding at the basolateral domain facilitates the spread of virus into subepithelial tissues and gaining access to the peripheral blood (18, 19, 21). The differential budding behavior of wild-type and F1-R viruses may explain, in part, why infection by wild-type virus is localized in the respiratory tract whereas infection by the F1-R mutant virus becomes systemic, presumably by spreading to distant organs via the blood.

3.4. Nucleotide sequence

By comparative nucleotide sequence analysis of the F gene of the host range mutants (ts-f1 and F1-R), six mutations were revealed in the F gene, two in the M gene, none in the HN gene, one in the NP gene of ts-f1, one in the P gene of F1-R, and one in the L gene of F1-R. Of significance is the predicted amino acid substitutions in the F protein at residues 115 (Ser to Pro) and 116 (Arg to Lys) at the site of cleavage of the F protein. One mutation was found in a revertant, T-5, derived from F1-R that was no longer pantropic. It was at residue 115 (Pro to Ser), which represents the mutation in the protein involved in the cleavability of the protein. This also rules out the possible role of the other mutations found in the F, P, and L genes of F1-R for pantropism.

3.5. Determinants of pantropism

Two determinants of pantropism have been suggested, the primary determinant of proteolytic cleavage and the secondary one as bipolar budding. One of the distinguishing phenotypes of F1-R was the abnormal migration of the M protein in SDS-PAGE gels. The protein migrated faster indicating a structural change in the M protein of F1-R. Two mutations in the M protein were at residues 128 (Asp to Gly) and 210 (Ile to Thr). These mutations have been postulated to cause bipolar budding of F1-R. Additionally, polarized transport of proteins in various epithelial cells depend upon the cytoskeletal system (22). It was shown that wild-type Sendai virus in polarized MDCK cells depend on intact microtubules, whereas in F1-R infected cells, the microtubules were disrupted (23).

To clarify which gene or mutations in F1-R was responsible for the disruption of microtubules leading to the altered budding phenotype, MDCK cells containing the F and M genes of wild-type and F1-R were established. Expression of the mutated F1-R M protein resulted in the formation of giant cells, bipolar transport of the F protein, and in the disruption of the microtubular network. This leads to the impairment of cellular polarity, bipolar transport of the F glycoprotein, and bipolar budding of the virus (24). The M protein plays an important role in the assembly process by binding the cytoplasmic tails of the glycoproteins with the nucleocapsid which is composed of the genomic RNA and NP, P, and L proteins (25).

Additional mutants with phenotypes similar to F1-R were derived from wild-type virus. This was to provide further evidence whether specific mutations in the F protein are solely responsible for the drastic change in infectivity, whether specific mutations in the M protein are responsible for bipolar budding, and whether bipolar budding of Sendai virus is a cofactor for pantropism.

Protease activation mutants that cleaved the F protein and mutants that bud bipolarly were isolated. Mutants that bud bipolarly, mutants with protease activation activity, and mutants with both phenotypes were characterized. Nucleotide sequence analysis of the F and M genes revealed deduced amino acid substitutions in the F and M proteins that were different from those of the respective proteins of F1-R, T-5 revertant of F1-R, and wild-type virus. The mutants did not cause systemic infections in mice and they did not disrupt the microtubules in MDCK cells (26).

These findings support the premise that one mutation (residue 115) in the F protein and two mutations (residues 128 and 210) in the M protein are the specific mutations required for the systemic infection of F1-R, and proteolytic cleavage of the F protein and bipolar budding attributed to disruption of microtubules (26) must function in concert for the systemic infection.

4. PERSPECTIVES

The determinants of organ tropism and for pantropism of the protease activation mutant, F1-R, are proteolytic cleavage and bipolar budding, attributed to mutations in the F and M proteins, respectively. Proteolytic cleavage permits the virus to undergo multiple cycles of replication in the lungs, the primary site of infection. Bipolar budding facilitates the systemic spread of the virus via the peripheral blood to distant organs where the virus again undergoes mutiple cycles of replication. This results in the systemic infection. Wild-type Sendai virus infection is restricted to the lungs whereas F1-R causes systemic infection. We propose that another determinant may be involved is in the enhanced disruption of the microtubules (26). Further studies are required to determine the mechanism by which the disruption of the microtubules occurs and the role in the pathogenesis of the pantropic mutant, F1-R.

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Send correspondence to: Dr N.L. McQueen, Department of Microbiology, California State University, Los Angeles, Los Angeles, California, 90032-8201, Tel: 81-3-5285-1169 , 323 343 2034, or 323 343 2036, Fax 81-3-5285-1169 or 323 343 6451, E-mail: mtashiro@nih.go.jp,jseto@calstatela.edu, nmcquee@calstatela.edu

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