

Review

New Insights into Measles Virus Brain Infections

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Measles virus (MeV) may persist in the brain, causing fatal neurodegenerative diseases, subacute sclerosing panencephalitis, and measles inclusion-body encephalitis. However, the mechanism of MeV propagation in the brain remains unexplained because human neurons affected by the diseases do not express the known receptors for MeV. Recent studies have revealed that certain changes in the ectodomain of the MeV fusion (F) protein play a key role in MeV spread in the brain. These changes destabilize the prefusion form of the F protein and render it hyperfusogenic, which in turn allows the virus to propagate in neurons. Based on crystal structures of the F protein, effective fusion inhibitors could be developed to treat these diseases.

MeV Infections in the Brain

MeV is a highly contagious human pathogen [1]. Although the use of effective live vaccines has greatly reduced the morbidity and mortality, measles still claims ~100 000 lives a year worldwide, and occasional outbreaks occur even in industrialized countries [2]. Specific antiviral agents are not currently available. While MeV usually causes self-limiting acute infection with symptoms such as fever, cough, and skin rash, it may persist in the central nervous system (CNS), causing intractable neurodegenerative diseases, subacute sclerosing panencephalitis (SSPE), and measles inclusion-body encephalitis (MIBE) [1]. SSPE typically occurs several years after acute infection. It is estimated that 6.5 to 11 cases per 100 000 cases of measles develop SSPE [3], and the risk of SSPE occurrence may be much higher in the children who are infected with MeV at younger ages [4]. In the affected brains, neurons and glia contain nuclear and cytoplasmic inclusion bodies (a hallmark of MeV infection), but no virus is seen budding from the surface of infected cells. The patients exhibit personality changes, myoclonus and dementia, and finally reach the late stage characterized by coma, vegetative state, and autonomic failure [5]. There is no effective treatment for the disease, although nonspecific antivirals (interferons, ribavirin, and inosine pranobex) have been used [5]. In immunosuppressed individuals, such as HIV(human immunodeficiency virus)-infected children, the disease with similar pathology and clinical manifestations, termed MIBE, may occur several months following exposure to MeV.

It is currently unknown why persistent MeV brain infections occur in rare cases. Importantly, human neurons, a main target affected by the diseases, do not express the known receptors for MeV, and the mechanism underlying MeV propagation in the brain remains unexplained. In this review, we describe recent developments in studies of the MeV fusion (F) protein, which shed new light on MeV infection in the brain and its possible control.

Properties of MeV

MeV, a member of the genus *Morbillivirus* in the family Paramyxoviridae, is an enveloped virus with a nonsegmented, negative-strand RNA genome (Figure 1A) [1]. Its genome has six genes

Highlights

Measles virus (MeV), in rare cases, persists in the central nervous system and causes fatal neurodegenerative diseases.

Human neurons do not express the known receptors for MeV (CD150 and nectin 4), and the mechanism of MeV propagation in the brain is unknown.

The specific changes in the ectodomain of the MeV fusion (F) protein, rather than the defects in the matrix protein and the cytoplasmic tail of the F protein, play a key role in MeV spread in the brain.

The changes in the ectodomain destabilize the prefusion form of the F protein and render it hyperfusogenic, which in turn allows MeV to propagate in neurons.

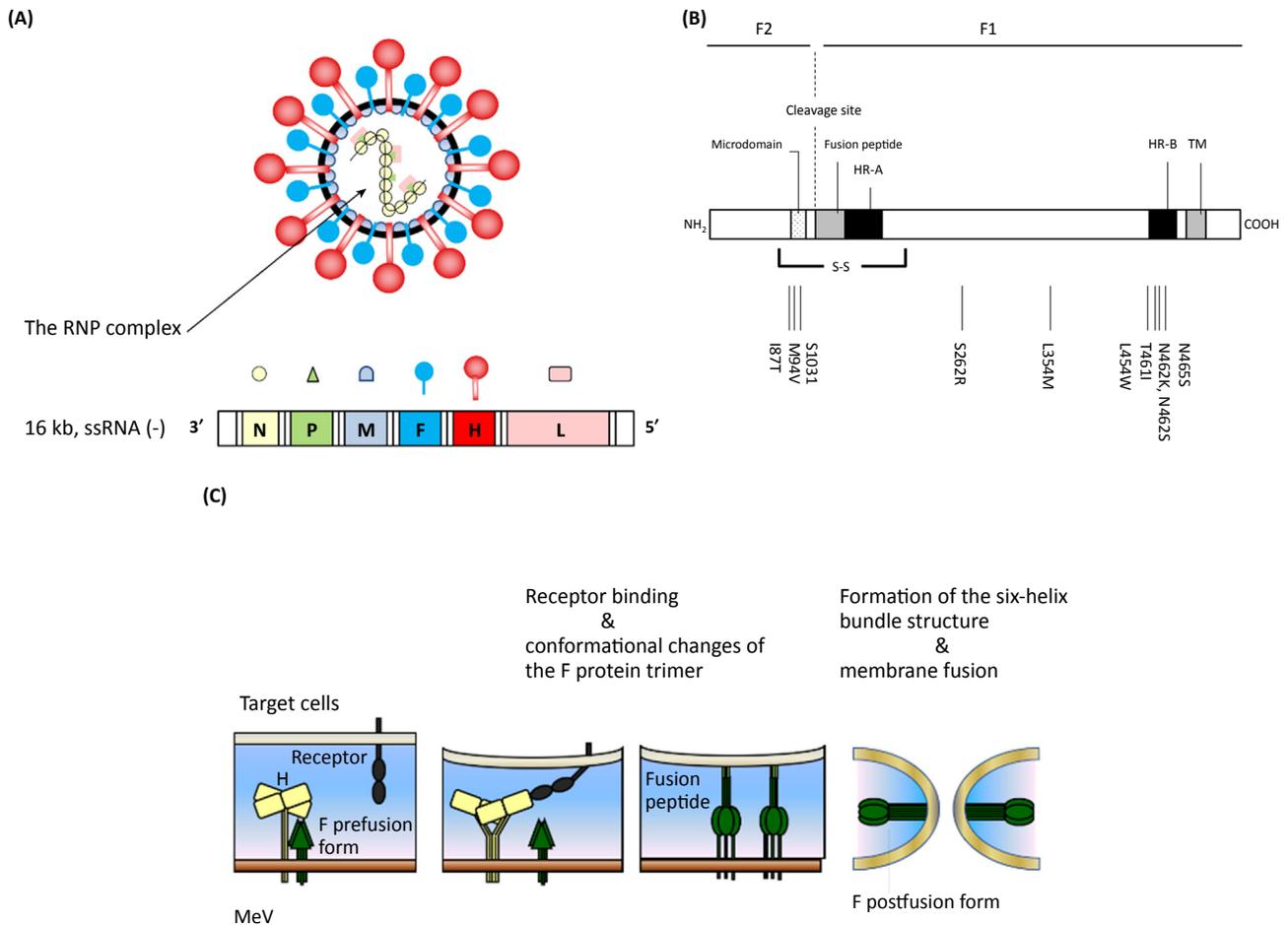
The putative neuronal receptor for MeV may be required for the MeV attachment protein to trigger destabilized F proteins.

Crystal structures of the F protein will serve as a structural template to develop more effective fusion inhibitors to treat MeV brain infections.

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Figure 1. Properties of the Measles Virus (MeV). (A) Schematic diagrams of the MeV particle (upper) and genome structure (lower). Viral genes are indicated by the same colors as their corresponding proteins. ss, single-stranded. (B) Schematic representation of the MeV F protein with amino acid substitutions destabilizing the prefusion form of the protein and enhancing its fusion activity. HR-A and HR-B, two heptad repeat domains. TM, transmembrane domain. The disulfide bond between the F₁ and F₂ subunits, the cleavage site, and the fusion peptide are also indicated. (C) MeV-mediated membrane fusion during virus entry. See text for details.

that encode the nucleocapsid protein (N), phosphoprotein (P), matrix protein (M), F protein (F), hemagglutinin (H), and large protein (L). The RNA genome encapsidated by the N protein forms the ribonucleoprotein (RNP) complex with the viral RNA-dependent RNA polymerase comprised of the L and P proteins. The two envelope glycoproteins have a role in receptor binding (H protein) and membrane fusion (F protein). The H protein exists on the envelope as a homotetramer, whereas the F protein occurs as a homotrimer [1,6,7]. The F protein is first produced as a nonfunctional precursor, F₀, that is cleaved by the host protease, furin, within the Golgi apparatus into the fusion-competent disulfide-linked mature protein composed of the F₁ and F₂ subunits (Figure 1B) [8]. The cleavage generates a new N terminal hydrophobic segment in the F₁ subunit, known as the fusion peptide. The M protein interacts with the RNP complex, as well as the cytoplasmic tails of the H and F proteins, and plays an important role in the assembly of virus particles.

MeV enters host cells by pH-independent membrane fusion at the cell surface (Figure 1C). It is thought that once the H protein binds a cellular receptor this causes conformational changes of the F protein trimer [7,9,10]. These conformational changes allow the insertion of the fusion peptide into the plasma membrane of the target cell and the interaction of the two heptad repeat (HR) domains, HR-A and HR-B, in individual F protein monomers, forming the six-helix bundle structure (Figure 1B,C) [8,11]. These structural rearrangements in the F protein (the prefusion to the postfusion form) bring the virus envelope and the cell membrane into very close apposition and destabilize them, leading to the fusion of the two lipid bilayers. While triggering of the F protein induces virus-to-cell fusion during virus entry, it causes cell-to-cell fusion between infected and neighboring cells, producing syncytia (giant multinucleated cells) later in infection [1].

The cellular receptors for MeV are the signaling lymphocyte activation molecule (SLAM, also called CD150) expressed on immune cells, and nectin 4 on epithelial cells [12–14]. Vaccine and laboratory-adapted strains of MeV (e.g., the Edmonston strain) can use ubiquitously expressed CD46 as an additional receptor, through amino acid changes in the H protein [10]. MeV, an airborne pathogen, first infects SLAM⁺ immune cells in the respiratory tract and spreads to the immune system throughout the body. At the later stages, MeV-infected immune cells transmit the virus to respiratory epithelial cells through nectin 4, and progeny viruses produced in the respiratory epithelia are released into the environment by coughing and sneezing [13,14].

‘Characteristic’ MeV Sequence Changes in SSPE and MIBE

Cell-associated, but not cell-free, MeVs may be isolated by coculturing brain tissues from SSPE and MIBE patients with susceptible cell lines. Many studies have determined MeV nucleotide sequences from virus isolates thus obtained or directly from brain tissues without virus isolation [15–25]. It is now well established that MeVs from SSPE and MIBE brains contain ‘characteristic’ changes in their genomes [1,26]. First, the M gene is highly mutated in almost all cases. During viral persistence, adenine-to-guanine or uracil-to-cytosine biased hypermutations often occur in the MeV genome, especially in the M gene [15,18]. In some instances, mutations in the P gene lead to an increase in the level of the read-through P–M mRNA, precluding the production of the M protein [17,27]. These changes cause defects in the M protein, resulting in the lack of virus particle formation, which may help persisting MeVs to evade neutralizing antibodies. Second, many persisting MeVs have mutations in the F gene, which cause the elongation or shortening of the cytoplasmic tail of the F protein [23,24]. The changes in the M protein and the cytoplasmic tail of the F protein affect the interaction between the two proteins, increase the surface expression level of the F protein, and enhance cell-to-cell fusion [28–31].

It is likely that these changes accumulate during persistence because the M protein and the cytoplasmic tail of the F protein are dispensable for the survival of MeV, and their defects are selectively advantageous for MeV propagation in the brain. Indeed, an isolate (SSPE-Kobe-1), derived from a patient shortly after onset of SSPE, had only a small number of changes in the M gene, compared with the field isolate from which the brain isolate likely originated [20]. The contribution of these changes to MeV propagation in the brain was strongly suggested by Cathomen *et al.* [28,29]. They generated the recombinant MeV based on the Edmonston strain lacking the M protein and that lacking the cytoplasmic tail of the F protein, and showed that these viruses induced enhanced cell–cell fusion in CD46⁺ cells *in vitro* and penetrated more deeply into the brain parenchyma of genetically modified mice (human CD46-transgenic and type I interferon receptor knock-out (IFNAR KO) mice) than the parental Edmonston strain.

Although these ‘characteristic’ changes of the MeV genome are probably involved in MeV propagation in the brain, one has to be cautious of the interpretation of the results obtained with the Edmonston strain and recombinant viruses based on it. The Edmonston strain uses CD46, in addition to SLAM and nectin 4, as a receptor [32–34], and CD46 is expressed on all nucleated human cells, including neurons [35]. Thus, CD46-using MeVs such as the Edmonston strain efficiently infect human neurons in a CD46-dependent manner. Indeed, a case with MIBE was undoubtedly caused by a vaccine strain [36]. However, epidemiological studies with sequence analyses have demonstrated that vaccine strains are not responsible for SSPE cases [3,37]. Importantly, human neurons express neither SLAM nor nectin 4 [38,39], and therefore wild-type strains of MeV can hardly infect human neurons. Bearing these facts in mind, recombinant MeVs based on the wild-type IC-B strain were generated and used to re-examine the effect of the lack of the M protein or the cytoplasmic tail of the F protein on MeV propagation. These mutant viruses induced larger syncytia and spread more efficiently, compared with the parental strain, in SLAM⁺ or nectin 4⁺ cells, but did not induce syncytia in SLAM[−] and nectin 4[−] cells (e.g., human neuroblastoma cell lines) [40]. These observations point to other molecular mechanisms that account for MeV propagation in the human brain.

Changes in the Ectodomain of the F protein

During analysis of MeV mutants obtained by *in vitro* passages, several single substitutions (I87T, M94V, S262R, L354M, N462K, N462S, and N465S) were identified in the ectodomain of the F protein that conferred on the F protein enhanced fusion activity [40,41] (Figure 1B, Table 1). These substitutions were largely located at the specific regions of the ectodomain of the F protein, which had been reported to affect membrane fusion, such as the microdomain preceding the cleavage site and the HR-B domain [40,42–46] (Figure 1B). When expressed together with the H protein, the F protein possessing one of these single substitutions not only

Table 1. Amino Acid Substitutions in the Ectodomain of the Measles Virus (MeV) F Protein That Render the Protein Destabilized and Hyperfusogenic

Substitutions	Fusion in SLAM ⁺ cells ^a at 37 °C	Fusion in SLAM ⁺ cells at 25 °C	Fusion in SLAM [−] nectin 4 [−] cells	Clinical conditions containing the substitution	Refs
Wild-type	+	−	−	Wild-type	[6,40,50]
I87T	+/++	ND	ND	No	[40]
M94V	++	+	+	SSPE	[6,22,40,42,72]
S262R	+++	+	+	No	[6,40]
L354M	++	+	+	No	[40]
L454W	+++	+	+	MIBE	[6,49]
T461I	+++	+	+	SSPE	[6,18,22,23,40,50,56]
N462K	+++	+	+	No	[6,23,40,42]
N462S	++	ND	ND	SSPE	[6,18,19,21,40,42]
N465S	++	ND	ND	SSPE	[6,18,19,21,40,42,73]
N465K	+++	+	+	SSPE	[6,23,40,42]
S103I/N462S/N465S	+++	+	+	SSPE	[6,19,21,40,50,56]
FΔ30 ^b	++	−	−	SSPE	[30,40]

ND, not done.

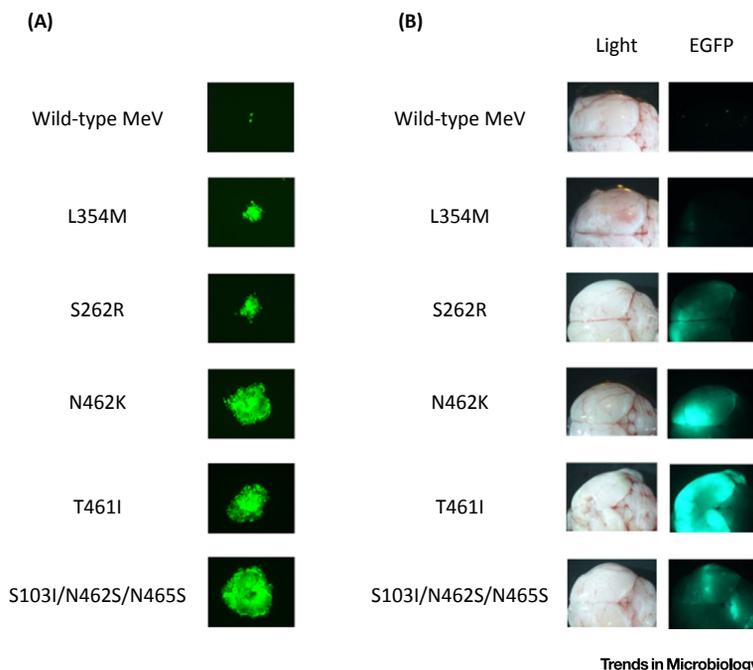
+, fusion observed; ++ enhanced fusion; +++, highly enhanced fusion; −, no fusion.

^aSLAM, signaling lymphocyte activation molecule.

^b30-amino-acid deletion in the cytoplasmic tail of the F protein.

induces larger syncytia in SLAM⁺ or nectin 4⁺ cells than the wild-type parental F protein, but causes cell–cell fusion even in SLAM[−] and nectin 4[−] cells (where no syncytia are induced with the wild-type F protein). Recombinant MeVs (based on the wild-type strain) possessing these mutant F proteins, unlike the parental virus, induce syncytia and propagate in SLAM[−] and nectin 4[−] cells (Figure 2A) and exhibit neurovirulence in suckling hamsters after intracerebral inoculation (Figure 2B). The mortality is correlated with the fusion activity of the viruses [40].

Within clinical isolates of MeV, F protein sequences are highly conserved [47]. For example, the IC-B strain (genotype D3) isolated in 1984 has the same amino acid sequence as the Edmonston B strain (genotype A) derived from the first MeV isolate in 1954. Figure 3 lists all sequences of the complete F protein from SSPE and MIBE brains available in the National Center for Biotechnology Information (NCBI) database. Notably, the F protein sequences from SSPE and MIBE brains show high levels of variations in the ectodomain, especially at the microdomain preceding the cleavage site and HR-B domain, in addition to the well-documented alterations in its cytoplasmic tail. Among many variations, T461I and S103I/N462S/N465S are found in five and two SSPE viruses, respectively. When coexpressed with the H protein, the F protein with the T461I or S103I/N462S/N465S substitution exhibits enhanced fusion activity in SLAM⁺ or nectin 4⁺ cells, and induces syncytia in SLAM[−] and nectin 4[−] cells (Figure 2A) [40]. Furthermore, the recombinant MeV possessing the F protein with the T461I or S103I/N462S/N465S substitution induces syncytia in SLAM[−] and nectin



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Figure 2. Effects of the Changes in the Ectodomain of the F Protein on Measles Virus (MeV)-Mediated Membrane Fusion (Syncytium Formation) and Neurovirulence. SLAM[−] and nectin 4[−] Vero cells (A) or suckling hamsters (B) were infected with enhanced green fluorescent protein (EGFP)-expressing recombinant MeVs (based on the wild-type IC-B strain) bearing the F protein with indicated substitutions. The cells were observed under a fluorescence microscope at 3 days after infection. The wild-type MeV and the mutant virus bearing the F protein with the L354M substitution did not kill hamsters, but the other recombinant MeVs killed the majority of infected hamsters within 7 days after inoculation. The brains of euthanized or dead hamsters were observed under a fluorescence stereomicroscope, and the spread of EGFP-expressing recombinant MeVs was examined. Light and EGFP images of the brains were photographed. See [40] for details. SLAM, signaling lymphocyte activation molecule.

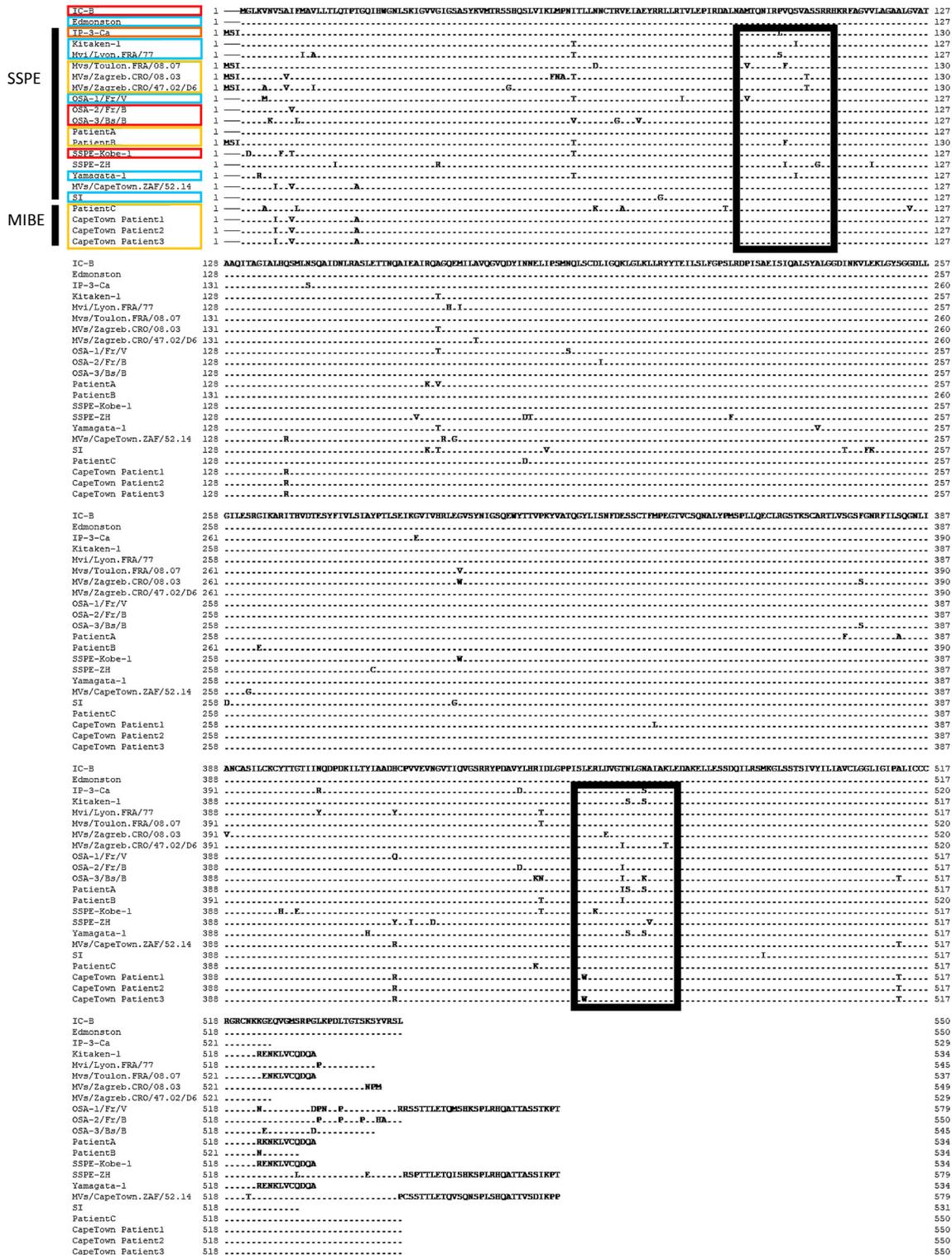


Figure 3. Alignment of Amino Acid Sequences of the Measles Virus (MeV) F Protein from SSPE and MIBE Brains, together with Those of the IC-B and Edmonston B Strains. The sequences are from IC-B (the wild-type strain) (NC_001498) [69], Edmonston B (Z66517) [70], IP-3-Ca (X16566) [71], Kitaken-1 (See figure legend on the bottom of the next page.)

4⁻ cells, and exhibits neurovirulence in suckling hamsters (Figure 2B) [40,48]. The F protein possessing the L454W variation found in MIBE patients is also shown to exhibit fusogenicity in SLAM⁻ and nectin 4⁻ cells [49]. Thus, the changes in the ectodomain of the F protein of SSPE/MIBE viruses can confer on the F protein the same phenotype as those of the MeV mutants obtained through *in vitro* passages. By contrast, the lack of the M protein or the deletion of the cytoplasmic tail of the F protein *per se* cannot provide the wild-type MeV with this property (see the previous section).

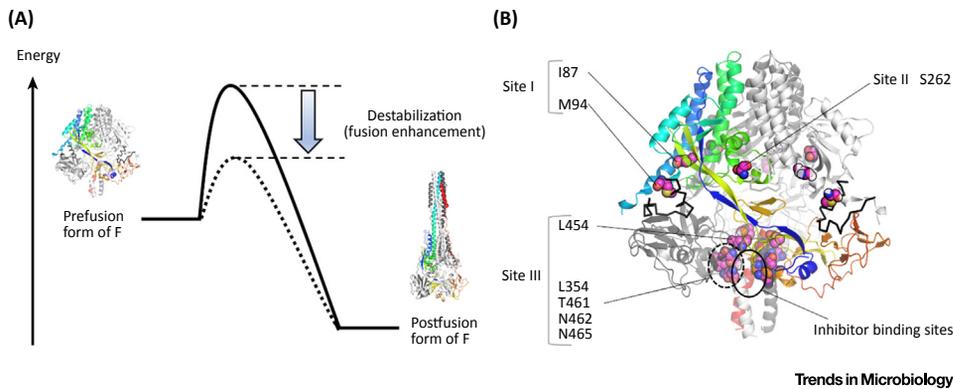
Thus, the hitherto overlooked changes in the ectodomain of the F protein appear to be critical for MeV to induce membrane fusion and propagate in SLAM⁻ and nectin 4⁻ cells such as neurons. When the wild-type MeV somehow establishes persistent infection, it does not likely propagate efficiently in neurons at first. However, some variants may emerge during persistence that have hyperfusogenic F proteins and propagate in neurons. With time, variants capable of better propagation in neurons may dominate and eventually cause the diseases. Since individual SSPE/MIBE viruses have many variations in the ectodomain of the F protein, all of these variations, as well as the changes in the M protein and the cytoplasmic tail of the F protein, may affect the overall fusogenicity of the F protein.

Destabilization of the F Protein Confers Hyperfusogenicity

How do specific changes in its ectodomain allow the F protein to exhibit enhanced fusion activity in SLAM⁺ or nectin 4⁺ cells and to induce syncytia even in SLAM⁻ and nectin 4⁻ cells? Expression levels of the F protein at the cell surface are not largely affected by them [40]. Instead, these changes in the ectodomain appear to decrease the conformational stability of the prefusion form of the F protein. The wild-type F protein, when coexpressed with the H protein, can induce membrane fusion in SLAM⁺ or nectin 4⁺ cells at 37 °C, but not at lower temperatures (e.g., at 25 °C). By contrast, the F protein containing these changes in the ectodomain can still induce membrane fusion at 25 °C (Table 1) [50].

In MeV-mediated membrane fusion, the energy required to overcome the kinetic barrier is provided by the conformational changes of the F protein (Figure 4A) [11,51]. Decreased stability of the F protein lowers the threshold level of activation energy so that the F protein would be more easily triggered. Interaction of the H protein with SLAM or nectin 4 at 37 °C triggers the wild-type F protein, but changes in the ectodomain of the F proteins lead to triggering at lower temperatures. Destabilized F proteins may also be triggered by the interaction of the H protein with molecules beyond SLAM and nectin 4 (see below). The similar destabilized, hyperfusogenic F protein mutants have also been described in other paramyxoviruses [52,53].

(SSPE 75) (AB453046) [21], Mvi/Lyon.FRA/77 (HM562894) [22], Mvi/Toulon.FRA/08.07 (AEF30358) [22], MVs/Zagreb.CRO/08.03/_SSPE (DQ227320) [16], MVs/Zagreb.CRO/47.02/[D6]_SSPE (DQ227318) [16], OSA-1/Fr/V (AF179434) [23], OSA-2/Fr/B (AF179436) [23], OSA-3/Bs/B (AF179439) [23], Patient A (X16567) [18], Patient B (X16568) [18], SSPE-Kobe-1 (AB254456) [20], SSPE-ZH (AB453185), Yamagata-1 (D10548) [19], MVs/CapeTown.ZAF/52.14 (KT851537), SI (JF791787.1) [25], Patient C (X16569), Cape Town Patient 1 (KC305657), Cape Town Patient 2 (KC305658), and Cape Town Patient 3 (KC305659). A dot indicates identity with the corresponding amino acid residue in the IC-B strain. The cell lines used for original virus isolation are shown with the colored open rectangle surrounding each strain name. Red, blue, and brown rectangles indicate B95a (SLAM⁺), Vero (CD46⁺), and BS-C-1 (CD46⁺) cells, respectively. An orange rectangle indicates that the sequence was determined by direct sequencing without virus isolation. The information regarding the cells used for virus isolation is not available for strains with no surrounding rectangle. Highlighted with a black rectangle are the regions preceding the cleavage site (first row) and corresponding to the N terminal part of the HR-B domain (fourth row). Most of the MeV mutants with destabilized hyperfusogenic F proteins obtained by *in vitro* passages were also found to have single amino acid substitutions in these two regions. SLAM, signaling lymphocyte activation molecule.



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Figure 4. Triggering Conformational Changes of the F Protein. (A) Destabilization of the F protein through specific substitutions in its ectodomain lowers the threshold level of activation energy required for initiating conformational changes of the metastable prefusion structure. This enables the H protein, upon interaction with the putative neuronal receptor, to trigger such destabilized F proteins, but not the wild-type F protein. (B) Amino acid residues (sphere diagrams) whose substitutions confer hyperfusogenicity on the F protein are shown on the crystal structure of the prefusion form of the F protein trimer viewed from the side (shown as a ribbon). These residues are clustered in three sites. Binding sites for fusion inhibitors AS-48 and FIP are also shown. Each monomer is colored in rainbow, light gray, or dark gray.

Recently, crystal structures of the prefusion F protein trimer alone and in complex with a small fusion inhibitor compound AS-48 [45,54], or a fusion inhibitor peptide (FIP) [55], were determined [6]. In the F protein structure, the ectodomain residues that confer hyperfusogenicity on the F protein after their changes from the wild-type sequence are clustered in three specific regions (termed 'sites I, II, and III' [6]) (Figure 4B). The 'site I' is located close to the fusion peptide, and the 'site II' is situated at the interface of three monomers. The 'site III' is the region connecting the head and stalk domains of the F protein and corresponds to part of the HR-B domain. These three sites likely play a role in maintaining the metastable prefusion structure and/or initiating the conformational changes upon triggering. The F protein structure has provided structural explanations for destabilization of the prefusion conformation caused by these changes in the ectodomain [6]. Among the three sites, the 'site III' appears to be most critical as many positions are changed in SSPE/MIBE viruses (Figure 3). Furthermore, 'site III' almost overlaps with the binding site for fusion inhibitors AS-48 and FIP (Figure 4B). These inhibitors presumably stabilize the metastable prefusion structure and prevent its conformational changes upon triggering. Thus, the 'site III' may represent a highly critical region regulating MeV-induced membrane fusion.

Recombinant MeVs bearing hyperfusogenic F proteins with the changes in the ectodomain exhibit stronger cytopathology (due to syncytium formation) and produced lower titers in SLAM⁺ or nectin 4⁺ cells, compared with the wild-type MeV [50]. Furthermore, these viruses spread efficiently in the brain, but not in the spleen of SLAM knock-in and IFNAR KO mice [50]. Thus, it appears that spread of MeV in the brain is aided by enhanced fusion activity, but spread is less in SLAM⁺ or nectin 4⁺ cells due to stronger cytopathology. This could have resulted in sequences of the F protein being highly conserved, without variations suggesting the hyperfusogenic phenotype, among MeV clinical isolates [47].

MeV Cell-to-Cell Spread between Neurons

One of the pathological features of SSPE/MIBE is the lack of syncytia in the affected brain [26]. The hyperfusogenic recombinant MeVs based on the wild-type strain also do not induce syncytia in human primary neurons [50] or postmitotic neurons obtained by treating the human

embryonal carcinoma cell line NTERA-2 cl. D1 (NT2) with retinoic acid [56], unlike in other SLAM⁺ and nectin 4⁺ cell lines. However, these hyperfusogenic viruses, but not the wild-type MeV, efficiently spread between human primary neurons or between NT2-derived neurons [50,56]. Confocal time-lapse imaging with NT2-derived neurons showed that the viruses spread from the first neurons infected along axons to connected neurons. The observation, combined with the finding that production of MeV particles is severely suppressed in NT2-derived neurons, suggests that hyperfusogenic viruses largely spread between neurons in a cell-to-cell manner. Since FIP blocks this MeV spread, membrane fusion mediated by the F protein is likely involved in the process [56]. Recently, the cell-to-cell contact-dependent spread of the wild-type MeV, without producing syncytia, has also been reported in nectin 4⁺ airway epithelial cells [57]. In the brain, other supporting cells and myelinated nerve fibers could block cell–cell contacts between neurons, and cell–cell contacts could be limited to small areas such as synapses. Thus, membrane fusion may occur only at synapses where transmission of the viral RNP complex takes place between neurons. These spatial arrangements could be why neurons do not form syncytia in SSPE/MIBE patients.

How hyperfusogenic F proteins are triggered for conformational changes remains to be determined. They may be nonspecifically triggered at the surface of infected neurons. Alternatively, there may be a specific neuronal receptor that interacts with the H protein. Some anti-H protein monoclonal antibodies blocked MeV infection dependent on the known receptors (SLAM and nectin 4) as well as the spread of a hyperfusogenic MeV between NT2-derived neurons [56]. One antibody inhibited the former but not the latter, while another antibody only weakly inhibited the former, but strongly blocked the latter. Although other explanations are possible, these results may suggest that, in addition to hyperfusogenic F proteins, the interaction of the H protein with a specific receptor is required for MeV spread between neurons. Thus, the identification of the putative neuronal receptor is a priority to fully understand the molecular mechanism of MeV spread between neurons. If the putative neuronal receptor is exclusively expressed at synaptic membranes, it would also explain why neurons do not develop syncytia. Furthermore, the restricted distribution of the receptor may imply that hyperfusogenic MeVs do not easily infect neurons, but once they do, they efficiently spread between them.

SLAM- and nectin 4-independent noncytolytic spread of canine distemper virus (CDV), another member of the morbilliviruses, between astrocytes has been reported, suggesting the existence of a third receptor for the virus [58]. But this CDV spread in astrocytes does not require hyperfusogenic changes of the F protein. MeV spread dependent on cell-to-cell contact has also been reported between mouse neurons [59] or rat hippocampal neurons [60], using the Edmonston strain-based recombinant MeV. The authors of these studies proposed micro-fusion at synaptic membranes as a mechanism of MeV spread [59–61].

Fusion Inhibitors as Therapeutic Agents for MeV Infections in the Brain

SSPE can be prevented by vaccination against MeV, but there is no effective treatment after its occurrence. Considering the importance of membrane fusion in MeV spread in the brain, the F protein may be a good target of antiviral agents to ameliorate the symptoms of SSPE and MIBE patients. Polypeptides derived from the HR regions of the F protein inhibit MeV-mediated membrane fusion [62,63], and their modifications such as lipid-conjugation has proved highly effective in protecting animal models from MeV infection [64–66]. FIP is a modified tripeptide originally designed based on the sequence of the F₀ cleavage site [55], whereas the small compound AS-48 was designed based on a model structure of the F protein [45,54]. Crystal structures showed that both AS-48 and FIP bind to the 'site III' of the ectodomain of the F

protein (Figure 4B) [6]. Importantly, AS-48 and FIP could suppress membrane fusion mediated by most of the destabilized, hyperfusogenic F protein mutants [6]. With crystal structures of the F protein alone and complexed with the inhibitors in hand, the structure-based design may be feasible to develop more effective inhibitors. On the other hand, MeV mutants resistant to AS-48 and/or FIP have been reported [42,67], and their mutations are located around the inhibitor-binding sites on the F protein [6]. In fact, one of the MeV mutants that arose in the presence of FIP was a virus bearing the F protein with the L454W substitution [67], which was also found in two MIBE patients. Combined use of different inhibitors would overcome the problems of potential resistance and selection of hyperfusogenic viruses.

Concluding Remarks and Future Perspective

We still do not know why MeV persists in the CNS, causing fatal neurological diseases in rare cases. The immunosuppressed state is a prerequisite for MIBE, and some host genetic factors are likely involved in the development of SSPE [68]. This line of study has been difficult to perform due to the paucity of the patients, but should be pursued using the newest technologies (see Outstanding Questions). On the other hand, we are beginning to uncover the mechanism of MeV propagation in neurons that lack the known receptors. As we have described in this review, destabilizing hyperfusogenic changes in the ectodomain of the F protein are essential for MeV spread in neurons (Figure 5, Key Figure). Notably, it is the F protein, but not the receptor-binding H protein, that changes during persistence to allow the virus to exhibit tropism for neurons. We envisage that the wild-type H protein interacts with the putative neuronal receptor, and then triggers hyperfusogenic F proteins but not the wild-type F protein. Whether this reasoning proves correct awaits the identification of the putative neuronal

Key Figure

A New Understanding of Measles Virus (MeV) Propagation in the Brain

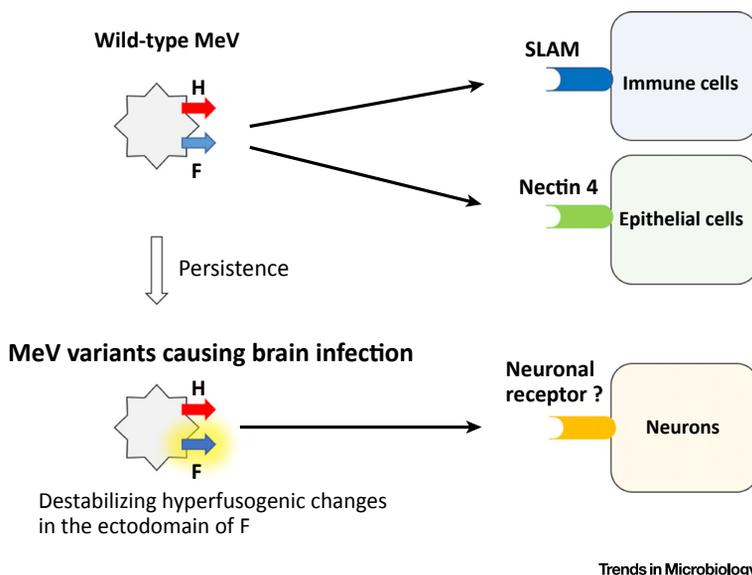


Figure 5. SLAM, signaling lymphocyte activation molecule.

Outstanding Questions

What allows MeV to persist in the brain in rare cases? Are any host genetic factors involved in the process?

The host immune response is evidently mounted because high titers of anti-MeV antibodies are detected in sera and cerebrospinal fluids of SSPE patients. Then, how can persisting MeVs evade the host immune response?

Is a specific receptor molecule on neurons required for triggering destabilized hyperfusogenic F proteins? If so, what is the neuronal receptor for MeV? Are orthologues of the human receptor molecule involved in MeV propagation in animal brains?

Why does the interaction of the H protein with the putative neuronal receptor trigger destabilized F proteins, but not the wild-type F protein? Does it differ quantitatively or qualitatively from its interaction with SLAM and nectin 4?

Does MeV spread between neurons indeed occur at synapses? If so, how is the MeV RNP complex transported to synapses despite the defect in the M protein? In cultured cell lines, the M protein binds to the RNP complex and brings it to the cell surface where MeV particle formation occurs, followed by budding.

Currently available fusion inhibitors possess only intermediate antiviral potency in cultured cells, with a low-to-submicromolar range of 50% inhibitory concentrations. Is it possible to develop more effective fusion inhibitors to treat MeV infection in the brain?

receptor. Furthermore, its identification would allow us to determine whether the same molecule is involved in the two properties uniquely exhibited by hyperfusogenic F proteins, fusogenicity in SLAM⁺ and nectin 4⁺ cell lines, and spread between human neurons. Finally, its identification is required to properly interpret the results from animal studies, as it is unknown whether orthologues of the human receptor molecule are used for MeV propagation in animal brains.

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