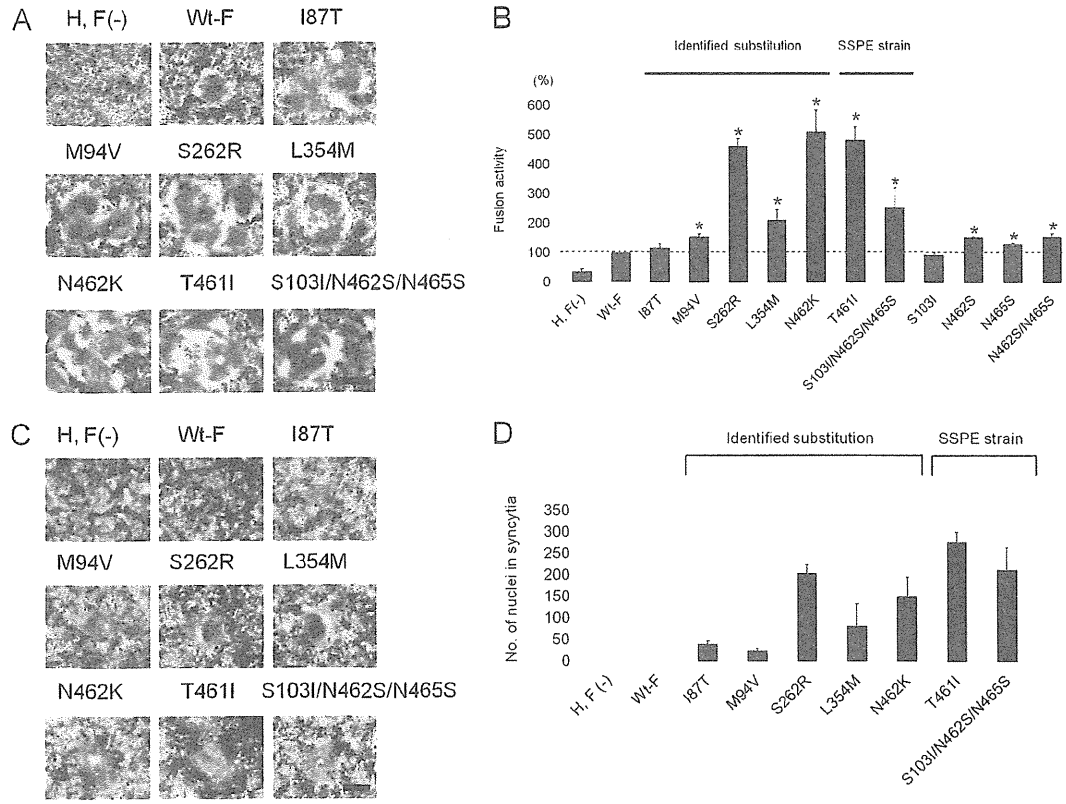

FIG 1 Amino acid substitutions in the F protein enhance cell-cell fusion in Vero/hSLAM and Vero cells. (A) Vero/hSLAM cells were transfected with expression plasmids encoding wild-type (Wt) or mutant F proteins with the indicated substitutions, together with the plasmid encoding the wild-type H protein. Only the H-protein expression plasmid was transfected in H, F(-). At 24 h posttransfection, the cells were stained with a Giemsa solution and observed under a light microscope. A representative syncytium is shown. Scale bar, 200 μm. (B) Quantification of cell-cell fusion in Vero/hSLAM-T7 cells. 293T cells were transfected with expression plasmids encoding the H protein and the indicated mutant F protein, together with the T7 polymerase-driven expression vector encoding luciferase. At 6 h posttransfection, transfected 293T cells were cocultured with Vero/hSLAM-T7 cells. After 18 h of incubation, cell-cell fusion was quantified by measuring the luciferase activity. Luciferase activity with the wild-type H and F proteins was set to 100%. The bars indicate the means and standard deviations (SD) for triplicate samples. An asterisk indicates a statistically significant increase compared with the wild-type F protein ($P < 0.05$). A one-tailed Student's t test assuming unequal variance was used to analyze the data. (C) Vero cells were transfected and observed as in panel A. (D) Quantification of cell-cell fusion in Vero cells. Fusion activity is shown as the number of nuclei present in syncytia per visual field with a 10× objective lens by averaging the numbers in nine visual fields (+SD).

tutions in this region (51). The N462K substitution is located in the HR-B domain. Mutant viruses resistant to fusion-inhibitory compounds contain substitutions at position 462 of the F protein, including the N462K substitution (47, 52). These three regions (the microdomain, cysteine-rich region, and HR-B domain) in the F protein have been implicated in controlling its fusion activity.

Substitutions found in the F proteins of multiple SSPE strains confer increased fusion activity. Although the amino acid sequences of the F protein are highly conserved among clinical isolates and vaccine strains (53), SSPE strains have many substitutions throughout the F protein. There are no apparently characteristic substitutions, but it is notable that most SSPE strains have substitutions in the microdomain, as well as the N-terminal region of the HR-B domain. We noted that the T461I and S103I/N462S/N465S substitutions are present in multiple SSPE strains and located in the fusion-controlling regions described above (Fig. 2). Using virus-like particles, Ayata et al. have reported that the neurovirulence of one SSPE strain (Osaka-2) is related to the ability of its F protein to induce syncytia in Vero cells, which is attributed to a single substitution (T461I) (54).

We examined whether the T461I and S103I/N462S/N465S substitutions confer enhanced fusion activity on the wild-type F protein. When expressed together with the wild-type H protein, the F protein containing the T461I or S103I/N462S/N465S substitutions induced larger syncytia in Vero/hSLAM cells than the wild-type F protein (Fig. 1A). The quantitative plasmid-mediated fusion assay also showed that these two mutant F proteins have 2.5- to 5-times-higher fusion activity in Vero/hSLAM cells than the wild-type F protein (Fig. 1B). The single substitution N462S or N465S slightly increased the fusion activity of the F protein, but the combined substitutions S103I/N462S/N465S additively enhanced fusion activity. The F protein containing the T461I or S103I/N462S/N465S substitutions also induced syncytia in receptor-negative (SLAM- and nectin 4-negative) Vero cells (Fig. 1C and D).

Surface expression of the mutant F proteins with enhanced fusion activity. To determine why these mutant F proteins exhibit enhanced fusion activity, their cell surface expression levels were examined (Fig. 3). All mutant F proteins had surface expression levels comparable to or slightly lower than that of the wild-type F protein, unlike the F protein with C68S/C195S substitutions, which is known to exhibit markedly decreased surface expression

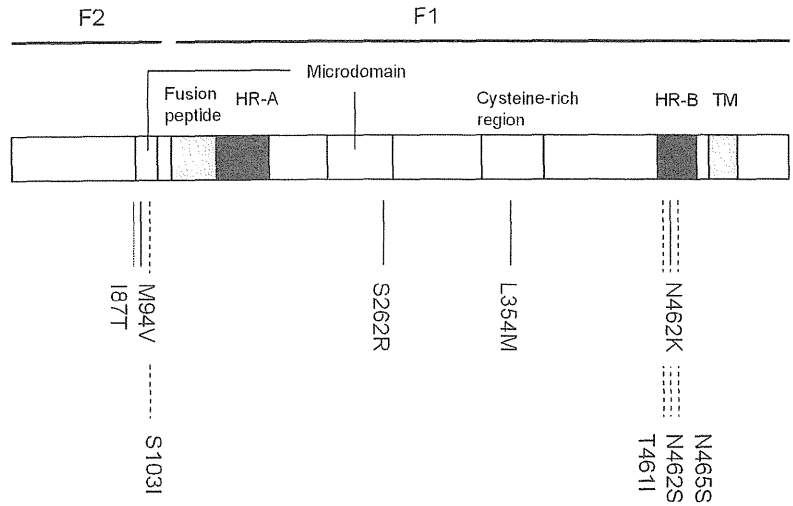

FIG 2 Locations of substitutions within the full-length F-protein structure. A schematic representation of the MV F protein and the positions of identified substitutions enhancing fusion activity (solid lines) are shown. The positions of fusion-enhancing substitutions found in multiple SSPE strains are also shown (dashed lines). HR-A and HR-B, heptad repeat domains; TM, transmembrane domain.

(48). The results indicate that the enhanced fusion activity of the mutant F proteins is due to their intrinsic fusogenicity, not to an increase in their surface expression levels.

Recombinant MVs with mutant F proteins induce syncytium formation in cells lacking both SLAM and nectin 4. To examine the effect of enhanced fusion activity of the F protein on MV pathogenicity, we selected five different F-protein substitutions (S262R, L354M, N462K, T461I, and S103I/N462S/N465S) that can induce high levels of fusion in Vero cells (Fig. 1D). These substitutions were incorporated into IC323-EGFP by using reverse genetics, producing five recombinant viruses expressing the wild-type H protein (without the epitope tag) and the respective mutant F proteins. The parental IC323-EGFP induced syncytia in nectin 4-positive cells, as well as in SLAM-positive cells. However, IC323-EGFP never produced syncytia in Vero cells lacking effective receptors (SLAM and nectin 4) for wild-type MV, although it occasionally infected single cells (Fig. 4A). In contrast, all five recombinant viruses with the mutant F proteins induced syncytia even in Vero cells.

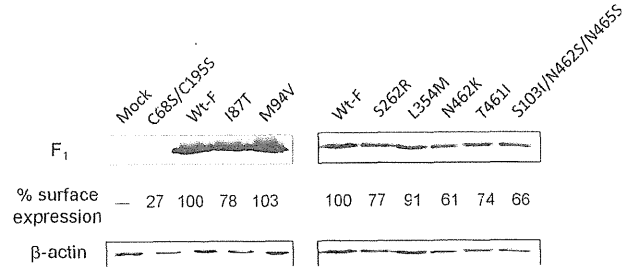

FIG 3 Expression levels of mutant F proteins at the cell surface. Biotinylated cell surface proteins separated by SDS-PAGE were reacted with antisera directed against the cytosolic domain of the F protein (detecting the F_1 subunit of the F protein). The upper left gel was exposed longer to allow visualization of the F protein with C68S/C195S substitutions, which has been known to exhibit markedly decreased surface expression. As a loading control, cell lysates were also subjected to SDS-PAGE and reacted with antibodies against β-actin. The surface expression level of each F variant was quantified and normalized to that of β-actin. The relative expression levels of F proteins are indicated below the respective bands (the normalized value of the Wt-F protein was set to 100). The data shown are representative of three experiments.

These recombinant viruses were also examined for the ability to infect the human neuroblastoma cell lines IMR-32 and SYM-1, which do not express SLAM and nectin 4. The absence of nectin 4 in these neuroblastoma cell lines was confirmed by reverse transcription (RT)-PCR to detect its transcripts (data not shown). Infection of IMR-32 and SYM-1 cells with IC323-EGFP resulted in some infected cells, but syncytia were never produced (Fig. 4B and C). Anti-human nectin 4 monoclonal antibody, which completely blocked infection of H358 cells with IC323-EGFP, did not affect that of IMR-32 and SYM-1 cells (data not shown). The five viruses with the mutant F proteins again induced syncytia in IMR-32 and SYM-1 cells, although the virus with the L354M substitution [IC-F(L354M)-EGFP] did not spread as efficiently as the other mutant viruses (Fig. 4B and C). Moreover, these five viruses induced larger syncytia in H358 cells (nectin 4-positive cells) than IC323-EGFP (data not shown).

Neurovirulence of recombinant MVs with mutant F proteins. To evaluate the neurovirulence of recombinant MVs with the mutant F proteins, we used a hamster model for MV infection with minor modifications (54). Six 10-day-old suckling hamsters were inoculated with IC323-EGFP or each mutant virus intracerebrally (10,000 PFU/brain). No hamsters inoculated with IC323-EGFP showed any symptoms for 4 weeks postinoculation (Fig. 5). In contrast, most of the hamsters inoculated with the recombinant viruses with the mutant F proteins, except IC-F(L354M)-EGFP, died or became moribund approximately 1 week postinoculation. Neurological signs (abnormal gait, convulsions, and continuous tremors) were observed 1 day before the hamsters became moribund. The L354M substitution has the weakest enhancing effect on the fusion activity of the F protein among the five substitutions (Fig. 1B and D and 4B and C). IC-F(L354M)-EGFP exhibited no neurovirulence in hamsters, while the other 4 mutant viruses showed high lethality (66 to 100%) (Fig. 5). Fusion activity in

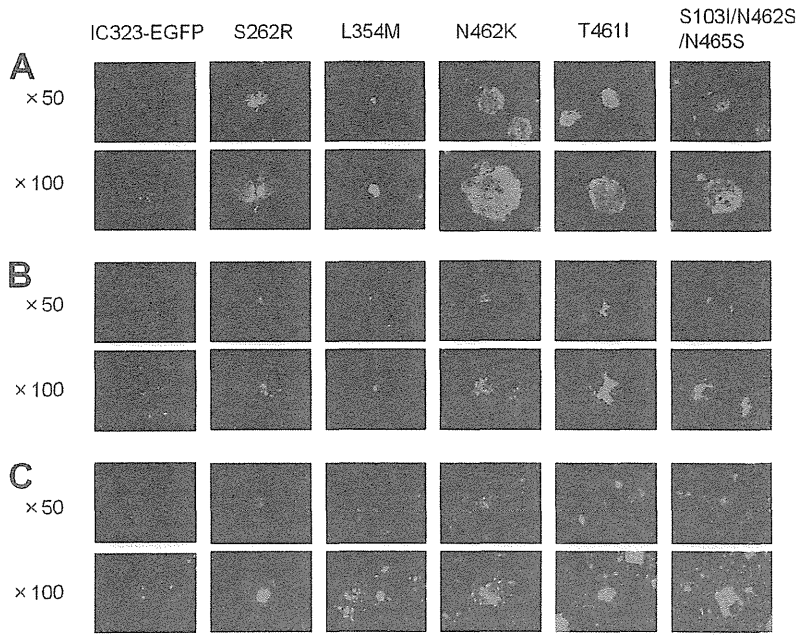

FIG 4 Syncytium formation in SLAM- and nectin 4-negative cells infected with recombinant MVs. Vero (A), IMR-32 (B), and SYM-1 (C) cells were infected with the parental recombinant MV expressing EGFP (IC323-EGFP) or its mutants expressing the F protein with the indicated substitutions at an MOI of 0.1. At 72 h after infection, EGFP fluorescence in infected cell monolayers was observed under a fluorescence microscope. Representative images are shown.

SLAM- and nectin 4-negative cells appeared to correlate well with the survival rate after virus challenge.

The brains were collected from moribund and dead animals, and the spread of EGFP-expressing recombinant viruses was examined under a fluorescence stereomicroscope (Fig. 6). Three hamsters were additionally inoculated with IC323-EGFP or IC-F(L354M)-EGFP and euthanized 6 days postinoculation for microscopic observation. While EGFP was not detected in the brains of hamsters inoculated with IC323-EGFP, IC-F(L354M)-EGFP was found to spread locally in the cerebrum. In sharp contrast, hamsters inoculated with the other 4 mutant viruses showed strong EGFP expression widely in their brains. After the observation, the brains were cocultured with Vero/hSLAM cells to recover viruses from hamsters inoculated with the mutant viruses. No amino acid substitution was found in the H, F, and M proteins of any recovered mutant virus compared with those of the virus used for challenge (data not shown), excluding the possibility that some mutations newly acquired *in vivo* account for the observed phenotype.

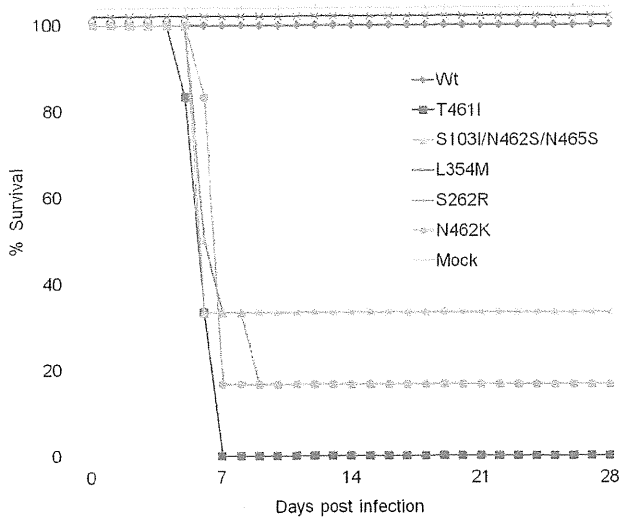

FIG 5 Mortality of hamsters after intracerebral inoculation with the mutant viruses exhibiting enhanced fusion activity. Six 10-day-old hamsters were infected with 10^4 PFU of wild-type MV (IC323-EGFP) (Wt), MV-F(T461I) (T461I), MV-F(S103I/N462S/N465S) (S103I/N462S/N465S), MV-F(L354M) (L354M), MV-F(S262R) (S262R), or MV-F(N462K) (N462K). Mock-infected hamsters were inoculated with growth medium (Mock). The animals were monitored for 28 days.

After observation with a fluorescence stereomicroscope, some of the brains were also subjected to histopathological examination (Fig. 7). Inflammatory cell infiltration was observed widely in the cerebra of hamsters inoculated with recombinant viruses possessing the mutant F proteins, except IC-F(L354M)-EGFP. In some lesions, monocytic or histiocytic cells predominantly invaded the inflamed sites, whereas the infiltration of lymphoid cells was observed in others. Severe lesions were found, especially in the cerebral cortex (Fig. 7A, B, and E) and the pyramidal layer and dentate gyrus of the hippocampus (Fig. 7C, D, and F). In these lesions, nuclear fragmentation, indicative of apoptotic cell death, was also observed. Syncytial giant cells were not observed in any brain samples. In contrast, few changes were found in the brains of hamsters inoculated with IC323-EGFP or IC-F(L354M)-EGFP. Immunohistochemical analysis (data not shown) confirmed that the region where EGFP was detected also expressed MV proteins. Moreover, EGFP colocalized with an astrocyte marker, GFAP, or with a neuronal marker, NeuN, showing that the mutant viruses infected astrocytes, as well as neurons, although the number of EGFP-positive astrocytes was smaller than that of EGFP-positive neurons.

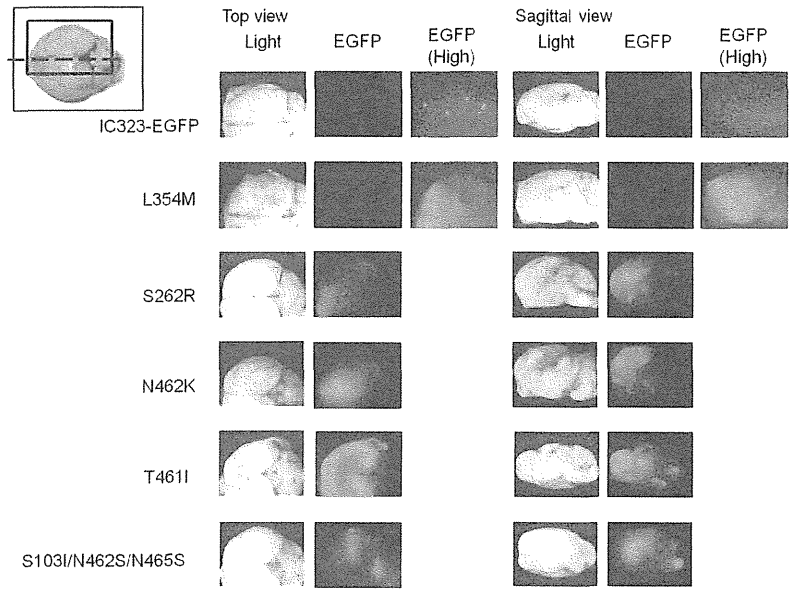

FIG 6 Spread of EGFP-expressing recombinant viruses in the brains of infected hamsters. The brains of euthanized or dead hamsters were observed under a fluorescence stereomicroscope, and the spread of EGFP-expressing recombinant viruses was examined. Light and EGFP images of the brains were photographed, and top and sagittal views (indicated in the inset by a rectangle and a dotted line, respectively) are shown. For IC323-EGFP and IC323-F(L354M)EGFP, high-sensitivity EGFP images (with a longer exposure time) are also shown.

Taken together, the results indicate that neurovirulence is correlated with the severity of lesions induced by viruses [IC323-EGFP and IC-F(L354M)-EGFP versus recombinant viruses with the other mutant F proteins].

Infection of SLAM- and nectin 4-negative cells with the recombinant MV lacking the M protein or the cytoplasmic domain of the F protein. To compare the contributions of fusion-enhancing mutations in the extracellular domain of the F protein to SLAM- and nectin 4-independent MV infection with that of defects of the M protein, Vero and IMR-32 cells were infected with the recombinant virus lacking the M protein or the cytoplasmic domain of the F protein. First, the recombinant MV lacking the M protein (M-less MV) was generated by using reverse genetics, and the absence of the M protein was confirmed in virus-infected cells.

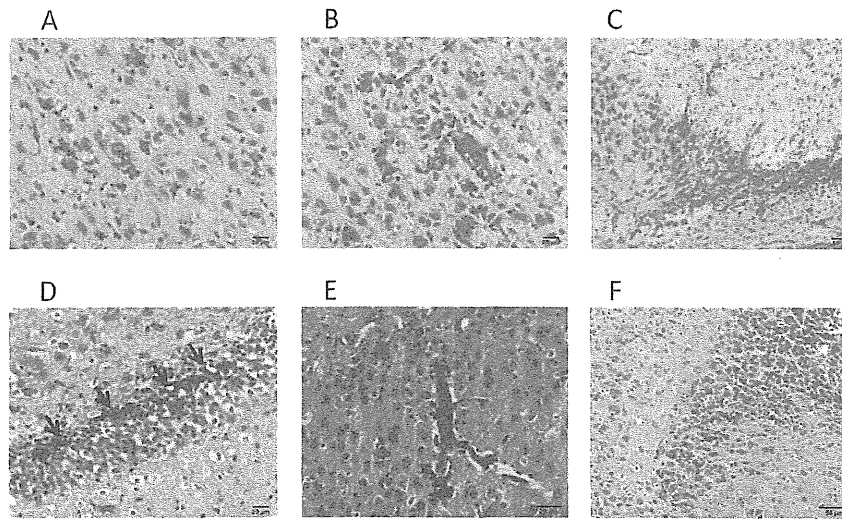

FIG 7 Histopathological examination of the brains of infected hamsters. The sections from the brains of infected hamsters were stained with HE. (A and B) Lesions in the cerebral cortexes of hamsters inoculated with IC-F(S262R)-EGFP. Invasion of monocytic or histiocytic cells into the parenchyma (A) or perivascular space (B) is shown. (C and D) Lesions in the hippocampuses of hamsters inoculated with IC-F(T461I)-EGFP. Infiltration of monocytic or histiocytic cells into the pyramidal layer (C) or dentate gyrus (D) of the hippocampus is shown. The arrows indicate nuclear fragmentation. (E and F) Lesions in the brains of hamsters inoculated with IC-F(N462K)-EGFP. Invasion of lymphoid cells into the cerebral cortex (E) or the pyramidal layer of the hippocampus (F) is shown.

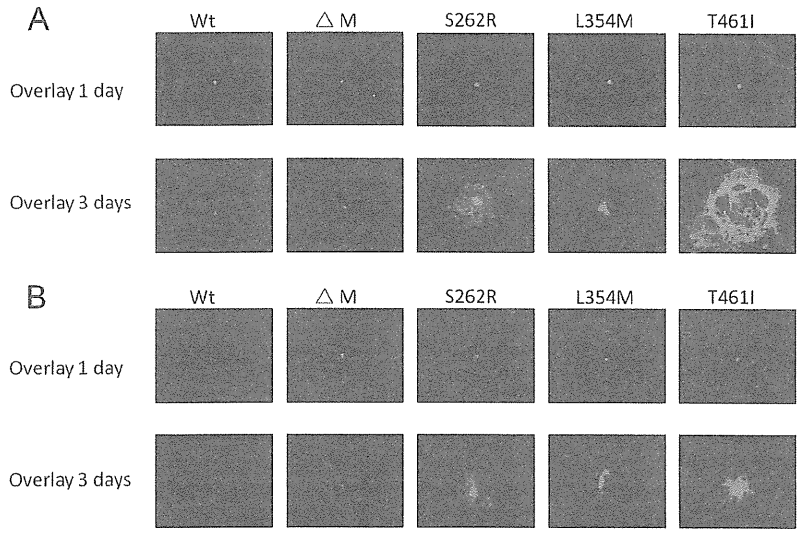

FIG 8 Limited fusogenic activity of the recombinant virus lacking the M protein in SLAM- and nectin 4-negative cells. IC323-EGFP, mutant viruses expressing the F protein with the indicated substitutions, and M-less MV (IC323-ΔM-EGFP) were rescued by using reverse genetics. BHK-T7/9 cells used for virus rescue were detached by trypsin digestion at 24 h after transfection with full-length-genome plasmids, and the preparation containing 200 EGFP-positive cells was overlaid onto a confluent monolayer of Vero (A) or IMR-32 (B) cells. At 1 or 3 days after overlay, EGFP fluorescence was observed under a fluorescence microscope. Representative images are shown.

Consistent with a previous report (37), M-less MV induced larger syncytia in Vero/hSLAM cells than wild-type MV (data not shown). As M-less MV could reach only low titers, it was not possible to perform infection of SLAM- and nectin 4-negative cells at sufficiently high multiplicities of infection (MOIs). Instead, BHK-T7/9 cells used for rescue of the recombinant M-less MV were detached by trypsin digestion and overlaid onto Vero or IMR-32 cells. Unexpectedly, apparent syncytium formation was not observed in either cell line (Fig. 8). Although small syncytium-like cells were occasionally observed in the cells cocultured with the rescued M-less MV, they were much smaller than syncytia formed by recombinant MVs possessing the mutant F proteins that were similarly rescued and processed (Fig. 8) (syncytia were observed 3 days, but not 1 day, after overlay). Moreover, expansion of these small syncytium-like cells was not found, even when the cells were observed 7 days after overlay.

Second, we examined the recombinant MV carrying the F protein lacking the cytoplasmic tail (IC323-EGFP-FΔ30) (39). Although we found that IC323-EGFP-FΔ30 induced larger syncytia in SLAM-positive cells than wild-type MV (39), the virus did not induce syncytia in Vero and IMR-32 cells (Fig. 9A and B). In addition, 8 hamsters were inoculated with IC323-EGFP-FΔ30, and 3 of them were euthanized 6 days postinoculation for microscopic observation (Fig. 9C). EGFP was detected in the brains of hamsters inoculated with IC323-EGFP-FΔ30 or IC323-EGFP only when observed in a high-sensitivity mode (with a long exposure time). The spread of IC323-EGFP-FΔ30 was locally restricted in the cerebrum, although it was more widespread than that of IC323-EGFP. The remaining 5 hamsters were examined daily for clinical symptoms of infection for 4 weeks postinoculation. No hamsters showed any symptoms, and EGFP was not detected in the brains of the hamsters at 4 weeks postinoculation (data not shown). The recombinant M-less MV could not be tested for *in vivo* infection experiments because of its low titers.

DISCUSSION

In this study, we have demonstrated that recombinant MVs bearing the mutant F proteins with enhanced fusion activity induce cell fusion in SLAM- and nectin 4-negative cells and exhibit neurovirulence in hamsters, unlike the parental wild-type MV. The mutations introduced into the F proteins of these recombinant viruses are (i) those found in multiple SSPE strains (T461I and S103I/N462S/N465S) and (ii) those not found in SSPE strains (S262R, L354M, and N462K). IC-F(L354M)-EGFP, possessing the least enhanced fusion activity among these viruses, spread locally in the brains of inoculated hamsters but did not cause lethality. These results suggest that enhanced fusion activity is one of the major determinants for MV neurovirulence and that MV must have a certain level of fusion activity in cells lacking both SLAM and nectin 4 in order to exhibit neurovirulence.

From the genetic study of SSPE strains, it has been thought that defects of the M protein play a crucial role in MV neuropathogenicity (55, 56). While cumulative mutations in the M protein may lead to the lack of virus particle formation and escape from host immune responses, the deletion of the M protein can also enhance membrane fusion (37). In addition, the cytoplasmic domain of the F protein, which interacts with the M protein, is elongated or shortened in some SSPE strains (34–36), and its deletion has been shown to enhance cell-cell fusion (38, 39). These fusion-enhancing effects were thought to be important for MV spread in the CNS. However, the effects of the deletion of the M protein or the F-protein cytoplasmic domain on MV fusion activity have been studied in SLAM- or CD46-dependent infection, but not in SLAM- and nectin 4-independent infection. Since SLAM and nectin 4 (receptors used by wild-type MV) were found to be scarcely expressed in the human brain (26, 57, 58), the relevance of the above-mentioned fusion-enhancing mutations in the M and F proteins should be reexamined in the context of SLAM- and nec-

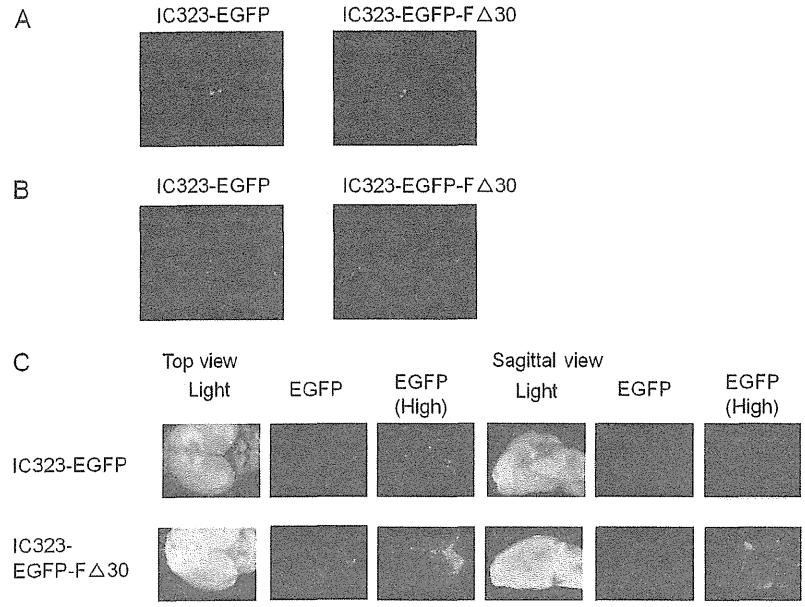

FIG 9 Infection with the recombinant MV with the F protein lacking the cytoplasmic tail (IC323-EGFP-FΔ30). SLAM- and nectin 4-negative Vero (A) and IMR-32 (B) cells were infected with IC323-EGFP or IC323-EGFP-FΔ30 at an MOI of 0.1. At 72 h after infection, EGFP fluorescence in infected cell monolayers was observed under a fluorescence microscope. Representative images are shown. (C) Spread of IC323-EGFP or IC323-EGFP-FΔ30 in the brains of infected hamsters. The brains of euthanized hamsters were observed as described in the legend to Fig. 6. High-sensitivity EGFP images with longer exposure times are also shown.

tin 4-independent infection. In fact, we could not detect the expression of nectin 4 in human neuroblastoma cells used in the present study, and anti-nectin 4 antibody did not block inefficient infection of these neuroblastoma cells with wild-type MV. In addition, Zhang et al. reported that other human neuroblastoma cell lines hardly express nectin 4 (59). Importantly, several whole-transcriptome analyses showed that the expression level of nectin 4 mRNA in the human brain is extremely low. These transcriptome data are accessible at the databases Gene Expression Atlas (http://www.ebi.ac.uk/gxa/) and RefExA (http://157.82.78.238/refexa/main_search.jsp). In contrast, it was reported that nectin 4 is expressed in the dog brain and is involved in the neurovirulence of canine distemper virus, which is closely related to MV and belongs to the genus *Morbillivirus* (60). The difference in neuropathogenicity between MV and canine distemper virus may be explained by the species difference in the tissue distribution of nectin 4.

Interestingly, neither M-less MV nor the recombinant MV carrying the F protein lacking the cytoplasmic tail (IC323-EGFP-FΔ30) induced apparent syncytia in Vero and IMR-32 cells (Fig. 8 and 9), although their fusion-enhancing effects were evident in SLAM-positive cells (37–39). In addition, IC323-EGFP-FΔ30 spread only locally in the brains of inoculated hamsters, unlike recombinant viruses with mutations in the extracellular domain of the F protein (Fig. 9). It is known that the M protein interacts with the cytoplasmic domains of viral envelope glycoproteins, as well as the viral RNP complex. Defects of the M protein, including those caused by deletion of the M protein or the cytoplasmic domain of the F protein may affect the structure and/or stability of viral envelope glycoproteins, leading to fusion enhancement in SLAM-, nectin 4-, or CD46-dependent infection. Moreover, the M protein inhibits MV RNA synthesis through its interaction with the viral RNP complex (via direct interaction with the N protein) (61). Thus, defects of the M protein may also increase the production of envelope glycoproteins. However, these effects caused by defects of the M protein are not enough to induce cell-cell fusion in cells lacking SLAM and nectin 4. In contrast, recombinant MVs with mutations in the extracellular domain of the F protein caused cell-cell fusion even in SLAM- and nectin 4-negative cells. Since the surface expression levels of these mutant F proteins were not affected significantly (Fig. 3), the intrinsic fusogenicity of these F proteins must be increased to induce cell-to-cell fusion in cells lacking SLAM and nectin 4.

In MV entry and virus-mediated cell-cell fusion, binding of the H protein to a cellular receptor triggers a series of conformational changes of the F protein, leading to membrane fusion. Thus, wild-type MV usually does not infect SLAM- and nectin 4-negative cells, nor does it induce syncytia in them. However, it is known that MV can infect various cultured cells (including neuronal cells and Vero cells) independent of known receptors, albeit at low efficiencies (100- to 1,000-fold lower than that of SLAM-dependent infection) (44). This inefficient infection produces solitary infected cells but does not induce syncytia. This presumably occurs because the F protein may sometimes be activated when the H protein interacts with an unidentified "inefficient receptor(s)." A previous study reported that the affinity of the H protein for a receptor had little impact on virus attachment, but it is nevertheless a key determinant of infectivity and cell-to-cell fusion (62). The expected low affinity of the H protein for the inefficient receptor might lead to inefficient infection but fail to induce cell-to-cell fusion (62). Certain substitutions in the extracellular domain of the F protein, especially the microdomain and HR-B domain, may decrease threshold levels for fusion triggering by the H pro-

tein, resulting in syncytium formation even in SLAM- and nectin 4-negative cells (via the inefficient receptor).

Notably, enhanced fusion activity of the F protein may be detrimental to the virus, as it can result in stronger cytopathogenicity and decreased virus production in SLAM-positive cells (43). This may be a reason why the F protein is highly conserved among clinical isolates and vaccine strains. However, the situation may be different in the CNS, where there are few efficient receptors available for wild-type MV and enhanced fusion activity allows the virus to spread via cell-to-cell fusion. Indeed, we demonstrated that MVs possessing enhanced fusion activity spread widely in the cerebra of hamsters lacking effective MV receptors, unlike the wild-type MV. Syncytial giant cells were not present in the brain samples as examined histopathologically, although viral proteins and GFP were detected widely in the cerebrum, including the cerebral cortex and hippocampus, by immunohistochemistry. This is consistent with the clinical observation that syncytia are not detected in the brains of SSPE patients (63). Recombinant MVs having the mutant F proteins were found to infect not only neurons, but also astrocytes (data not shown). The cell-cell contacts between these cells may be limited to small areas, such as synapses, and may be mostly hindered by other supporting cells and myelinated nerve fibers. This spatial arrangement may be a reason why neuronal cells do not form syncytia in MV-infected brains.

Recently, Seki et al. reported that an SSPE-derived strain (SI) uses CD46 and exhibits reduced fusion activity (45). The ability to use CD46 as a receptor, rather than enhanced fusion activity, may be a critical determinant for the strain's neuropathogenicity. It is possible that the ability to use the CD46 receptor has been acquired during virus isolation. In fact, many SSPE strains, including the SI strain, were isolated with Vero cells (27, 28, 64). Shingai et al. have reported that SSPE strains isolated with SLAM-positive cells do not utilize CD46 as a receptor (65). At any rate, the use of the CD46 receptor may be another strategy for MV to spread in the CNS, although its occurrence *in vivo* should be established by isolating more SSPE strains by using SLAM-positive (or nectin 4-positive) cells.

The present study indicates that even single-amino-acid substitutions (including those thus far unreported among SSPE strains) in its extracellular domain can confer enhanced fusion activity on the F protein, thereby allowing the virus to exhibit neurovirulence. Indeed, many SSPE strains have substitutions at different positions of the extracellular domain of the F protein, especially in the regions critical for controlling fusion activity, the microdomain and HR-B domain. Since nucleotide sequences of the F gene have been determined from only a limited number of SSPE strains, analysis of more SSPE strains, especially those isolated in SLAM-positive cells, may reveal unidentified mutations of the F gene, including those found in our study.

In the present study, we used 10-day-old suckling hamsters, as 3-week-old weanling hamsters were not susceptible to the viruses, unlike the prior report using virus-like particles (54). This suggests that the maturity of the host immune system is also important for MV neuropathogenicity in hamsters. At present, we do not know how MVs with mutant F proteins infect hamster neurons and astrocytes. It should be noted that receptors in hamsters may not be the same as the molecules used by MV in human patients with SSPE. Furthermore, in humans, acquisition of enhanced fusion activity due to mutations in the extracellular domain of the F protein may be a late event following several years of persistent MV infection in the CNS. Once acquisition of enhanced fusion activity occurs, viruses may spread effectively in the brain and cause SSPE. On the other hand, by using recombinant MVs with enhanced fusion activity, we can observe neurovirulence in hamsters within ~7 days.

Fusion-inhibitory reagents that efficiently block paramyxovirus-mediated fusion *in vitro* are available (66–69), and some of them are effective even for *in vivo* infection (70). Thus, blocking MV-mediated fusion with these reagents has the potential to be a good therapeutic approach for SSPE. To test these fusion-inhibitory reagents, the hamster model used in this study would be useful, and the development of effective therapy targeting MV-mediated fusion is the next step of the current study.

In conclusion, our data indicate that fusion-enhancing mutations in the extracellular domain of the F protein can cause SLAM- and nectin 4-independent cell fusion. Since human neuronal cells are mainly SLAM and nectin 4 negative, enhanced fusion activity may be one of the major determinants of MV spread and pathogenicity in the CNS, independently of defects of the M protein.

ACKNOWLEDGMENTS

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IASR

日本における麻疹ウイルス流行株の変遷　2009～2012

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WHOは、麻疹が排除されている状態を、「適切なサーベイランス体制の下で、ある特定の地域において、そこに常在する麻疹ウイルスによる麻疹症例が１年間以上存在しないこと」と定義しており1)、排除の認定には麻疹症例数を減少させるだけでなく、原因となった麻疹ウイルスの解析も求めている。わが国では国立感染症研究所、地方衛生研究所（地研）、保健所を中心としたRT-PCR法による麻疹検査体制を構築してきた2)。導入した当初は地研へ搬送される検体数が少なかったが、2011年、2012年には麻疹検査診断例の約40％が地研におけるRT-PCR法を利用していた。それに伴い、流行する麻疹ウイルスのゲノム情報も蓄積されてきている。

麻疹ウイルス遺伝子型の推移

2006～2012年に検出された麻疹ウイルスの遺伝子型の推移を図１に示す。2006～2008年の流行時には遺伝子型D5（バンコク型）のウイルスが主流であった3,4)。このD5型ウイルスが現在、日本における常在麻疹ウイルスとされている。2009年以降はD5型に代わって海外に由来すると考えられる他の遺伝子型のウイルスが増加してきている。2009年にはD5型（沖縄３例）、タイ帰国者からのD9型（山形）、インドとの関連が考えられたD8型（沖縄）5)のウイルスが検出された。2010年には中国からH1型２例（札幌、茨城）、インドからのD8型１例（横浜）、インドからのD4型１例（札幌）、フィリピンからのD9型16例（愛知、三重）、D5型１例（千葉）が報告された。このD5型ウイルスが日本で最後に報告されたD5型である。また、D9型ウイルスはフィリピンから４回にわたり日本に持ち込まれたが、系統樹解析では３つに分類されている6,7)。2011年はD4型が57例、D9型が49例、D8型が６例、G3型が２例報告されている。D4型は東京を中心とした首都圏、大阪、兵庫、山梨、新潟、広島で報告された。首都圏（東京47例、神奈川５例）ではイギリス、フランス、ドイツ等とのリンクが明らかな４件以外は疫学的な関連が不明な弧発症例（以下散発例）であったが、これら首都圏、ならびにスペイン（山梨）、フランス（大阪、兵庫）からの帰国者より検出されたD4型ウイルスのN遺伝子上の遺伝子型決定部位450塩基の配列は、１塩基の変異があった１株（東京）を除きすべて同一であった8)。一方、ニュージーランドからの帰国者（新潟）と散発例（広島）から検出されたD4型ウイルスはそれらと異なる配列であった。D9型のウイルスはフィリピン、カンボジア、シンガポールとスリランカ、グルジア、インドネシア、タイ、マレーシア等に渡航歴のある患者から検出されているが、これらの多くは異なる遺伝子配列をもっていた。D8型ウイルスはオーストラリア、タイ、バングラデシュおよびベトナムへの渡航歴を持つ患者から検出されている。また、インドネシア帰国者からG3型ウイルス２例（千葉、鹿児島）が報告されている。2012年はD8型ウイルスが45例、D9型が９例、D4型が６例、H1型が７例報告されている。D8型は2011年末に報告された成田空港勤務者から広がったと考えられる６例（千葉）、愛知から渡航歴のない小児から広がったと思われる24例（散発例とそこからの集団発生）、さらに岐阜３例（散発例とその家族）、山梨２例（散発例）が報告されたが、これらの遺伝子配列はすべて同一であった9,10)。これと同じ配列を持つD8型ウイルスは近年、欧州、アメリカ、オーストラリア、中東、インド等世界各地域からも報告されている。その他にタイ（東京、宮崎）、タイおよびカンボジア（東京）帰国者からもD8型ウイルスが報告されている。D9型はフィリピン帰国者（岡山、兵庫）、散発例（東京、千葉、栃木）からの報告があるが、岡山、兵庫、栃木の配列は異なっていた。D4型ウイルスはベトナム（東京）、パキスタン（富山）、英国とフランス（大阪）からの帰国者、ならびに２件の散発例（千葉、東京）から報告されているが、大阪の

株は2011年の欧州由来D4株と同じ配列であった。H1型では、福島で台湾由来と考えられるアウトブレイクがあったが、過去に報告されたH1型株とは異なる配列を持っていた11)。他に中国帰国者（千葉）と散発例（東京）からもH1型が検出されている12)。

従来の常在麻疹ウイルスと考えられていた遺伝子型D5型は２年間以上、検出されていない。しかし、新たに侵入した麻疹ウイルスの伝播が１年間以上継続すれば新たな常在株とされる。近年の日本の流行株の推移は欧州、東南アジア等の流行を反映している。小児だけでなく、国外へ行く機会が多い成人にも必要に応じた予防接種が勧められる。一方、収集した麻疹ウイルスの遺伝子解析の結果から、D4型やD8型のように海外の異なる場所や異なる時期に侵入したウイルスでも、遺伝子型決定部位の解析では鑑別ができない場合があることがわかってきた。また、都会を中心に散発例も多く報告されている。今後は１例１例の麻疹症例をウイルス学的、疫学的により丁寧に解析していくことが麻疹排除達成のために求められてくる。また、より詳細なウイルスの解析が可能となるウイルス分離の実施も望まれる。

謝辞：今回解析に用いた塩基配列は地研で実施された麻疹検査診断結果から得られたものです。麻疹検査診断をご担当いただいている地研ならびに保健所の皆様に深謝いたします。

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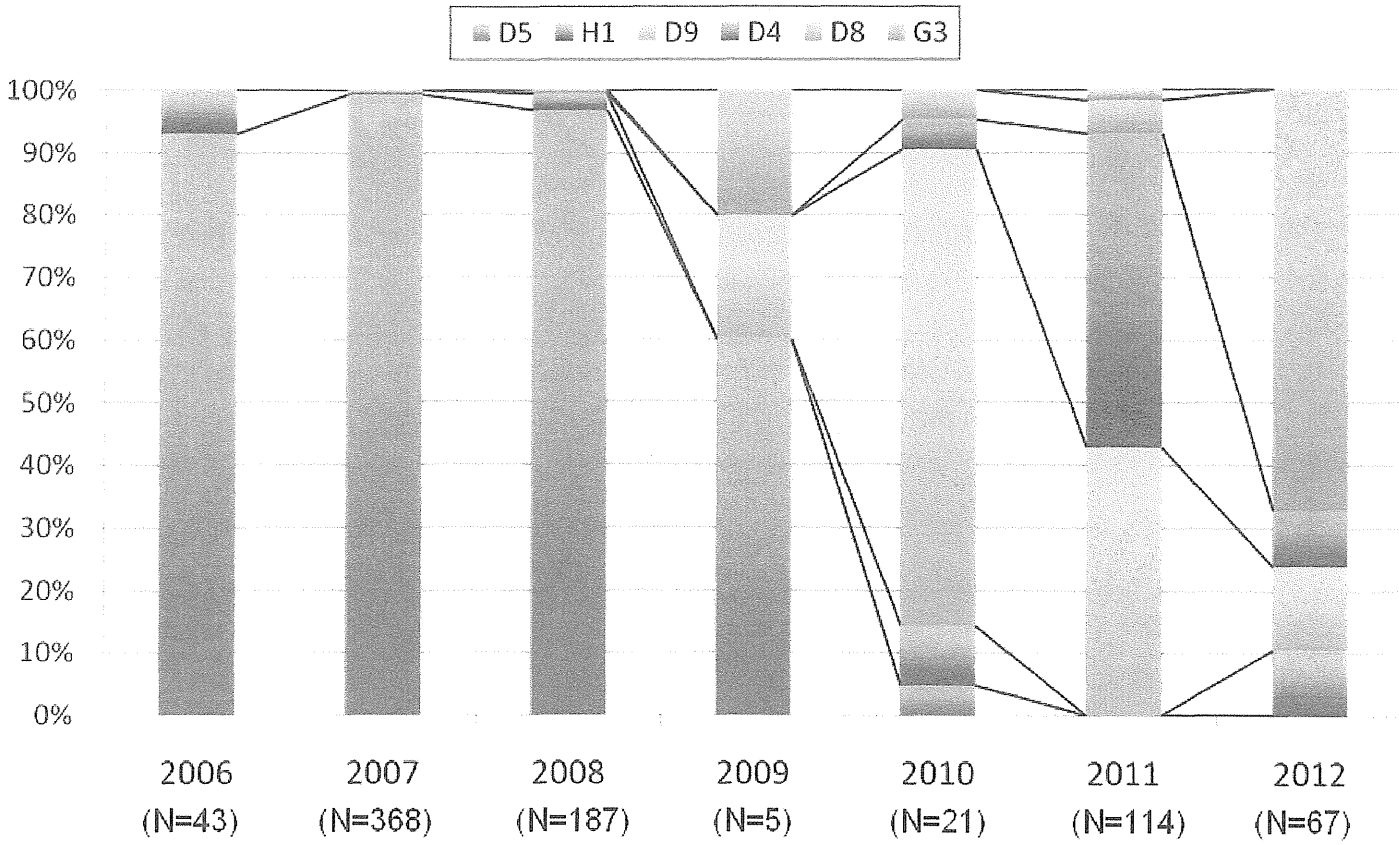

図1. 日本で検出された麻疹ウイルスの遺伝子型の推移

IASR
Infectious Agents Surveillance Report

IASR

沖縄県における麻疹発生ゼロの検証

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2010～2012年、沖縄県では３年連続で麻疹確定患者ゼロの状態を維持している（図１）。WHOは、Weekly Epidemiological Record（2010年12月）の中で、麻疹排除とは「質の高いサーベイランスのもとで、12カ月以上にわたり麻疹ウイルスの継続的な伝播がないこと」と定義している。今回、沖縄県の"麻疹ゼロ"状況を評価するため、WHOが示す麻疹排除達成の指標を適用し検討した。

まず、抗体保有状況として、血清疫学調査およびワクチン接種率を見ることが必要となる。前者において、2010～2012年に感染症流行予測調査で測定された麻疹PA抗体は、２歳以上のすべての年代で、抗体価1:16で95％以上陽性を示した（図２）。後者において、麻疹ワクチン接種率の第１期～第４期を指標とした場合、麻疹の排除目標である各期の接種率は95％以上には達しなかった（表１）。

次に、実験室診断による麻疹確定症例はゼロであることから、指標である人口100万人当たり１例未満を明らかに満たした。

また、質の高いサーベイランスの状況として、2010～2012年はWHOの示す以下の（１）～（４）の目標値を達成した。（１）年間の取り下げ症例について、2010年は1.8例／10万人でわずかに届かなかったが、2011年は2.7例、2012年は4.2例で、人口10万人当たり２例以上を満たした（図１）。（２）麻疹疑い例の80％以上から麻疹確定のための適切な検体採取が行われ、認可された実験室で検査されることについて、すべての麻疹疑い例で適切な検体採取が行われ検査診断がなされた（表２）。さらに、当実験室は、WHOに認可されている国立感染症研究所の麻疹IgM抗体測定Proficiency Testを受けており、要件を満たした。（３）集団発生の80％以上でウイルスの検出に適切な臨床検体が採取され、認定された実験室で検査されることについて、2006～2008年に発生した５件の集団発生では、すべての症例が検査診断され、患者間の疫学的リンクもすべて明らかにされた（IASR 28: 145-147, 2007＆30: 34-36, 2009＆30: 36, 2009）。2010～2012年には集団発生はなかった。認定された実験室での検査は上述の（２）で述べたとおり。（４）80％以上の麻疹疑い例で、症例届出後48時間以内に適切な調査が行われることについて、過去６年間において、95％以上の麻疹疑い例で症例届け出後０～２日（48時間）以内に適切な調査が行われていた（図３）。

以上のことから、2010～2012年のサーベイランスは適切であり、総合的に本県はWHOの求める麻疹排除（麻疹ゼロ）状態であると判断した。しかし、ワクチン接種率は79.1％（第４期）～94.2％（第１期）の範囲でいまだ95％を達成しておらず、今後の課題である。

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図1．麻疹確定例および人口10万人当たり否定例

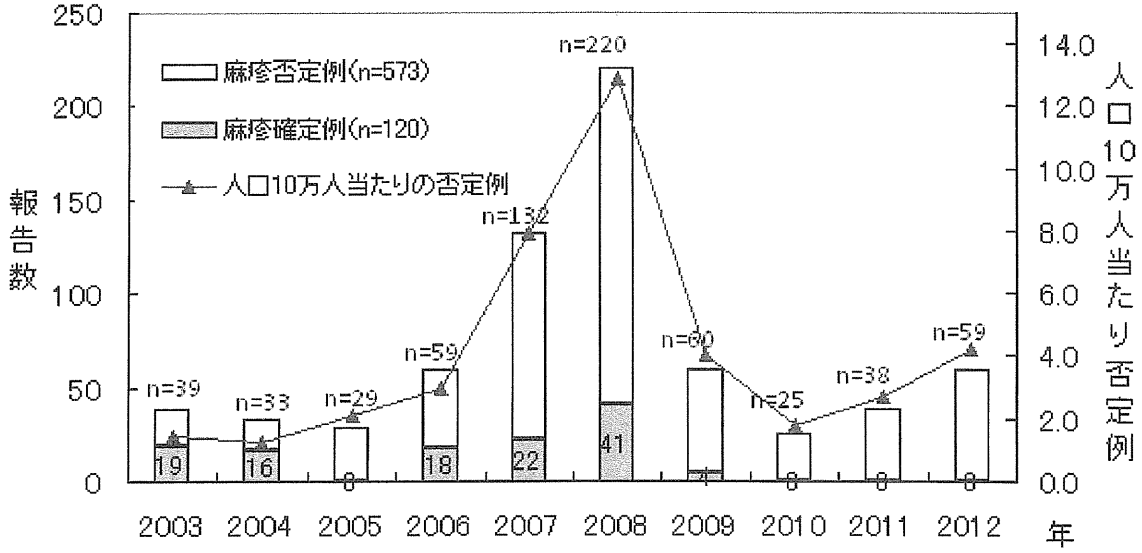

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図2. 年齢群別麻疹PA抗体保有状況(2010～2012年)

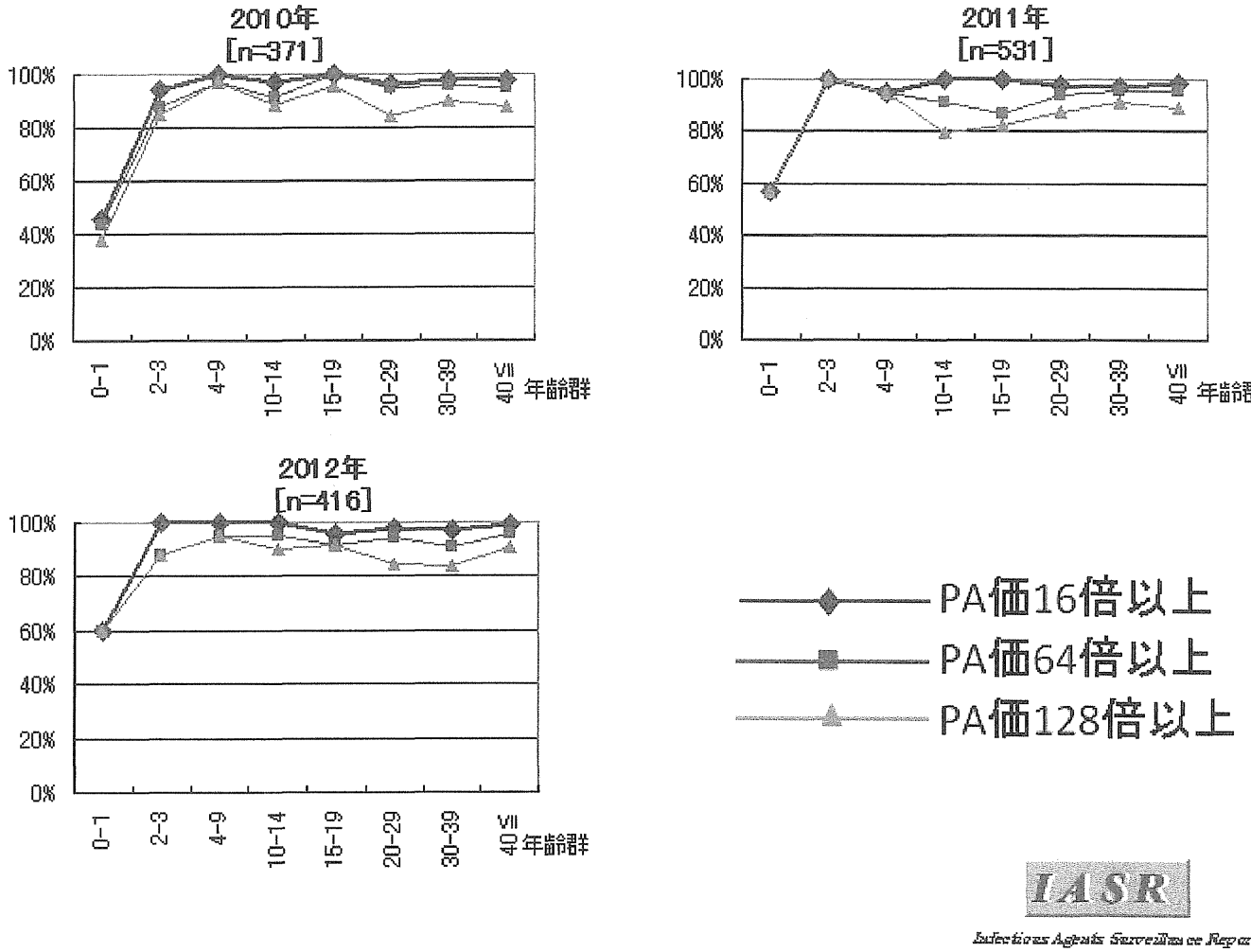

表1. 麻疹定期予防接種率(%)の推移

年度	第1期	第2期	第3期	第4期
2003	83.4			
2004	83.2			
2005	80.2			
2006	82.6	77.1		
2007	91.5	85.9		
2008	91.6	87.0	83.9	74.8
2009	91.5	88.6	84.4	76.5
2010	92.2	90.4	81.3	75.6
2011	94.2	91.9	82.2	79.1

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表2. PCRおよびIgM検査の実施率

年	報告数	検査診断	
		PCR(%)	IgM(%)
2003	39	24 (62)	18 (46)
2004	33	26 (79)	24 (73)
2005	29	27 (93)	11 (38)
2006	59	57 (97)	26 (44)
2007	132	125 (95)	45 (34)
2008	220	217 (99)	58 (26)
2009	60	60 (100)	40 (67)
2010	25	25 (100)	19 (76)
2011	38	38 (100)	34 (89)
2012	59	57 (97)	51 (86)
合計	694	656 (95)	326 (47)

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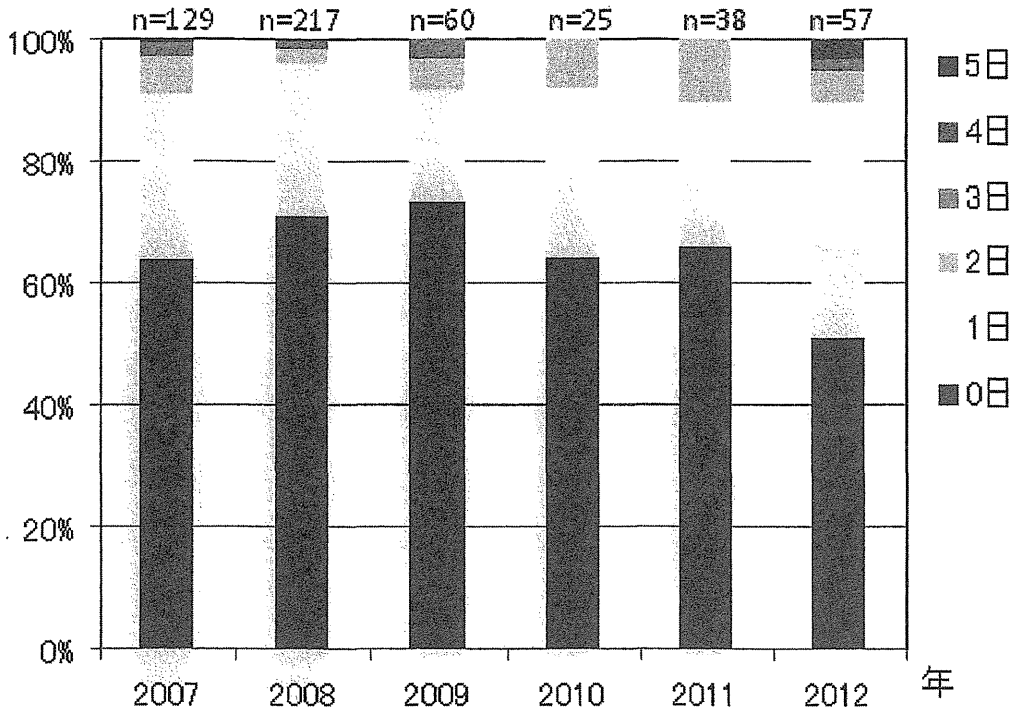
図3. 検体採取から検体搬入までの期間（2007～2012年）



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