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Role of Biased Hypermutation in Evolution of Subacute Sclerosing Panencephalitis Virus from Progenitor Acute Measles Virus

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We identified an acute measles virus (Nagahata strain) closely related to a defective virus (Biken strain) isolated from a patient with subacute sclerosing panencephalitis (SSPE). The proteins of Nagahata strain measles virus are antigenically and electrophoretically similar to the proteins of Edmonston strain measles virus. However, the nucleotide sequence of the Nagahata matrix (M) gene is significantly different from the M genes of all the acute measles virus strains studied to date. The Nagahata M gene is strikingly similar to the M gene of Biken strain SSPE virus isolated several years later in the same locale. Eighty percent of the nucleotide differences between the Nagahata and Biken M genes are uridine-to-cytosine transitions known as biased hypermutation, which has been postulated to be caused by a cellular RNA-modifying activity. These biased mutations account for all but one of the numerous missense genetic changes predicted to cause amino acid substitutions. As a result, the Biken virus M protein loses conformation-specific epitopes that are conserved in the M proteins of Nagahata and Edmonston strain acute measles viruses. These conformation-specific epitopes are also absent in the cryptic M proteins encoded by the hypermutated M genes of two other defective SSPE viruses (Niigata and Yamagata strains). Nagahata-like sequences are found in the M genes of at least five other SSPE viruses isolated from three continents. These data indicate that Biken strain SSPE virus is derived from a progenitor closely resembling Nagahata strain acute measles virus and that biased hypermutation is largely responsible for the structural defects in the Biken virus M protein.

Subacute sclerosing panencephalitis (SSPE) is a fatal degenerative central nervous system (CNS) disease that afflicts a small number of individuals as a sequel to acute measles (27, 30, 34). Viral agents that are isolated from patients with SSPE resemble acute measles virus strains, but they typically replicate in a cell-associated manner and produce no extracellular virus particles (8, 40). SSPE viruses are believed to be descendants of acute measles virus strains that initiate the original acute disease. The nucleotide sequences of viruses that cause SSPE and acute measles exhibit a large number of differences. However, the direct progenitors of SSPE viruses have never been isolated or identified. Therefore, the significance of the genetic differences and the purported evolutionary relationship between acute measles virus and SSPE viral agents remain to be established.

Recent studies revealed intriguing genetic divergence between known acute measles virus and SSPE virus strains. All the previously studied measles virus strains that cause acute infections, including Edmonston (20, 21), CAM (25, 35, 38, 39), and human-2 (Hu-2) (29), are closely related and can be grouped into a single class on the basis of their nucleotide sequence similarity (16). On the other hand, most SSPE viral agents differ considerably from these acute measles virus strains, and the nucleotide sequences of several independently isolated SSPE viruses are more closely related to one another than to those of the known acute measles virus strains (1, 16). This raises the possibility that SSPE viruses are derived from unidentified progenitor viruses distinct from currently known acute measles virus strains. If true, some of the mutations in the SSPE viral genes might actually represent genetic divergence between the true progenitors and the reference strains of acute measles virus. Resolving this question is crucial for distinguishing the true mutations acquired during chronic CNS infection from the natural genetic variations among different strains of measles virus and for understanding the functional significance of the mutations.

We report here the isolation and characterization of an acute neurotropic measles virus called Nagahata strain, which might be closely related to the direct progenitor of an SSPE virus. The matrix (M) gene of Nagahata strain measles virus diverges considerably from the M genes of previously studied acute measles virus strains but is strikingly similar to the M gene of Biken strain, a defective virus isolated later from a patient with SSPE in approximately the same locale (1, 37). Uridine (U)-to-cytosine (C) transitions constitute 80% of the nucleotide differences between the Nagahata and Biken viral M genes. These novel genetic changes, called biased hypermutation (6, 14, 41), are responsible for all but one of the missense mutations affecting the Biken M protein. These mutations destroy the conformation-specific epitopes conserved in the M proteins of acute measles virus strains. The Nagahata M gene also bears striking similarities to the M genes of several other SSPE viruses isolated from diverse locations. These results link Biken strain SSPE virus to Nagahata strain acute measles virus and suggest that biased hypermutation plays a significant role in the evolution of SSPE virus.

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MATERIALS AND METHODS

Viruses and cells. Nagahata strain measles virus was isolated in 1971 from a throat swab taken from a 5-year-old patient who had uncomplicated measles, in Toyonaka City, Osaka Prefecture, Japan. The virus was first isolated in primary human embryonic kidney cell cultures and subsequently passaged in human embryonic lung cells and African green monkey kidney (Vero or CV-1) cells. Edmonston strain measles virus (20) was kindly provided by C. Miller, University of Southern California School of Medicine. Comparative studies on Nagahata and Edmonston strains were performed with virus-infected CV-1 cells. Biken SSPE virus was maintained in chronically infected CV-1 (Biken-CV-1) cells. Cells were grown in minimal essential medium containing 10% newborn calf serum.

Protein analysis. Cells infected by different strains of acute measles virus or SSPE virus were starved for 1 h in methionine-free minimal essential medium until more than 50 to 80% of the cells showed cytopathic effects, and they were then labeled for 1.5 h with 35 μCi of [^{35}S]methionine (Dupont; NEN Research Products) per culture. Labeled proteins were immunoprecipitated and analyzed by electrophoresis in sodium dodecyl sulfate (SDS)-containing 10% polyacrylamide gel as described previously (1).

Antisera. The polyclonal GM antiserum was made against total native proteins of Nagahata strain measles virus by immunizing an African green monkey with CV-1 cells infected with Nagahata strain measles virus (38). The polyclonal M-BC antiserum was raised in rabbits against the denatured M protein of Edmonston strain measles virus expressed in bacteria from the *Bgl*II-*Cla*I fragment of the pcD-M2i Edmonston M cDNA clone (42). The polyclonal M-BBC antiserum was raised in rabbits against the denatured M protein of Biken strain SSPE virus similarly expressed from a corresponding fragment of the pTZ-BM1 Biken M cDNA clone (1, 2).

Cloned genes of measles and SSPE viruses. Full-length cDNA representing the M mRNA of Nagahata strain measles virus was cloned by a previously described procedure (1). Briefly, poly(A)$^+$ RNA was purified from Vero cells infected with an early-passage stock of Nagahata strain measles virus and annealed to an oligo(dT)-tailed pTZ18RX vector. The first cDNA strand was synthesized by reverse transcriptase. Synthesis of the second DNA strand was primed by a 20-nucleotide-long primer corresponding to the 5′ terminus of the Edmonston M mRNA. The resulting cDNA clones were circularized, transformed into *Escherichia coli* DH5, and screened by filter hybridization with a radiolabeled probe prepared from an Edmonston M cDNA. Full-length cDNA clones representing the M genes of Edmonston strain measles virus and Biken, Niigata, and Yamagata strain SSPE viruses have been described previously (1, 2, 41, 42).

Cloned genes were sequenced by the dideoxynucleotide-induced chain termination method (32) with Sequenase (United States Biochemicals).

In vitro expression of M genes. The M cDNA clones were linearized by cleaving at the *Hin*dIII site downstream of the M cDNA insert and transcribed into RNA by use of the T7 promoter in the pTZ18RX vector. Equal amounts of RNA were translated in rabbit reticulocyte lysates (Promega Biotec) in the presence of [^{35}S]methionine. Equal amounts of radiolabeled proteins were immunoprecipitated and analyzed by SDS-10% polyacrylamide gel electrophoresis (PAGE) (1).

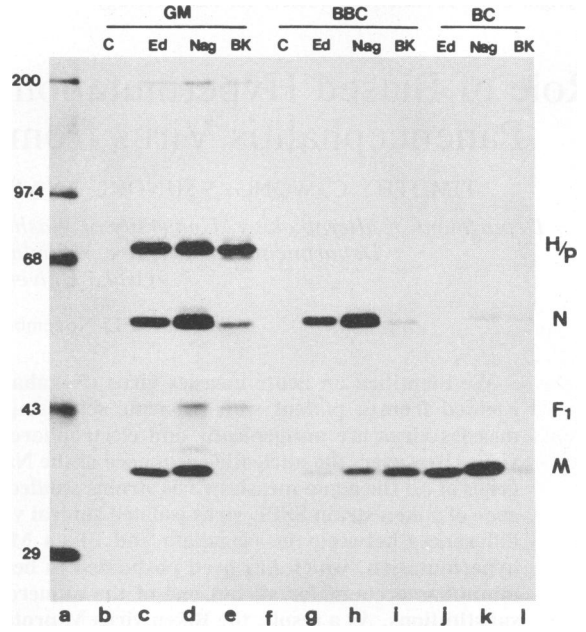

FIG. 1. Comparison of viral proteins produced by Nagahata, Edmonston, and Biken strains in vivo. CV-1 cells were infected with Edmonston or Nagahata strain measles virus (Ed and Nag, respectively) at a multiplicity of infection of 1. Cells were labeled with [^{35}S]methionine for 1.5 h at 25 h postinfection. CV-1 cells chronically infected with Biken virus (BK [2]) and uninfected CV-1 cells (C) were similarly labeled. Viral proteins were immunoprecipitated with GM antiserum against native Nagahata viral proteins (lanes b to e), M-BBC antiserum against the denatured Biken M protein (lanes f to i), or M-BC antiserum against the denatured Edmonston M protein (lanes j to l) and analyzed by SDS-PAGE. Molecular size standards are shown in kilodaltons (lane a).

RESULTS

Nagahata strain measles virus was isolated in 1971 from a 5-year-old patient who had uncomplicated measles in Toyonaka City, Osaka Prefecture, Japan. The virus replicated lytically in primary human embryonic kidney cells, human embryonic lung cells, and African green monkey kidney (Vero and CV-1) cells, causing extensive cytopathic effects typical of an acute measles virus.

Nagahata strain measles virus was neurovirulent. The original Nagahata viral isolate caused encephalitis in newborn and suckling hamsters but not in 4- to 6-week-old hamsters when inoculated intracerebrally. After four passages in suckling hamster brains, the virus exhibited more pronounced neurovirulence, causing encephalitis in both suckling and 4- to 6-week-old hamsters.

Characterization of Nagahata strain viral proteins. We compared the viral proteins in cells infected by the Nagahata strain with the viral proteins of Edmonston strain measles virus and Biken strain SSPE virus. An antiserum against the total proteins of Nagahata strain (GM serum) immunoprecipitated most of the intracellular proteins of Edmonston and Nagahata strains with equal efficiency (Fig. 1, lanes c and d). These included the nucleoprotein (N), hemagglutinin (H), M protein, F_1 subunit of the fusion protein, and phosphoprotein (P), which was not well resolved from H protein in some experiments. The GM antiserum also recognized most of the viral proteins of Biken strain SSPE virus except M protein (Fig. 1, lane e). A polyclonal antiserum against a peptide

synthesized from the Edmonston M gene (M-BC serum [42]) confirmed that the Nagahata M protein was antigenically related to the Edmonston M protein (Fig. 1, lanes j and k). This antiserum also detected a low level of the Biken M protein (Fig. 1, lane l).

To determine whether the Biken M protein had distinct antigenicity, we tested the same cell lysates with a polyclonal antiserum (M-BBC serum) made against a peptide synthesized from the cloned Biken M gene (see Materials and Methods). Indeed, the M-BBC antiserum readily immunoprecipitated the Biken M protein (Fig. 1, lane i). This Biken M-specific antiserum also reacted with the M proteins of Edmonston and Nagahata strains (Fig. 1, lanes g and h). However, since the Biken M protein had a much shorter half-life than the Edmonston M protein in vivo (1), the actual efficiency of the M-BBC antiserum in reacting with the M proteins of Edmonston and Nagahata strains was much lower than that with the Biken M protein. This conclusion was confirmed by immunological studies of in vitro-synthesized M proteins of the different virus strains (see Fig. 3). These results indicate that the M proteins of Nagahata and Edmonston strains are antigenically related and that the M protein of Biken strain SSPE virus has unique antigenic determinants.

The M-BBC antiserum also precipitated a 58,000-M_r protein which comigrated with the viral N protein from the virus-infected cells but not from the uninfected cells (Fig. 1, lanes f to i). That contaminating protein was also precipitated by the preimmune serum from the same rabbit as well as by antisera against other viral antigens from other rabbits. For example, the M-BC antiserum precipitated low levels of a similar protein (Fig. 1, lanes k and l) (42). Nonspecific activities against a similar protein were also reported in nonimmune rabbit serum as well as monoclonal antibodies against the F protein of Sendai virus (31). Whether that 58,000-M_r protein is the viral N protein or of cellular origin is presently unknown. In any case, boiling the cell lysates under denaturing conditions before immunoprecipitation by the M-BBC antiserum failed to remove the 58,000-M_r protein (22a), indicating that the contaminating protein did not associate with M protein as a complex.

Sequence similarity between the M genes of Nagahata strain measles virus and Biken strain SSPE virus. In light of the antigenic relatedness between the M proteins of the Nagahata and Edmonston strains, we expected the Nagahata M gene to be closely related to the Edmonston M gene. Surprisingly, when the M gene of the Nagahata strain was cloned and sequenced, it was found to diverge considerably from the M gene of the Edmonston strain. The Edmonston M gene (7, 42) was closely related to the M genes of other acute measles virus strains characterized, such as CAM-RB (14) and Hu-2 (17). The M genes of these acute measles virus strains differed by 0.5% or less from one another and could be represented by a consensus M sequence (Fig. 2, Cn). The Nagahata M sequence differed from this consensus sequence at 46 positions (Fig. 2, Na). Thus, the degree of divergence (3.1%) between the M genes of Nagahata and other acute measles virus strains was as high as between those other acute measles virus strains and some SSPE viruses (16).

Most importantly, the Nagahata M sequence exhibited striking similarities to the M gene of Biken strain SSPE virus (Fig. 2, Bk) (1). Thirty-six of the 46 nucleotides (78%) in the Nagahata M gene which differed from the consensus M sequence of other acute measles virus strains turned out to be identical to the corresponding Biken nucleotides (Fig. 2, Na and Bk, arrows; nucleotides 77, 245, 260, 278, 290, 297, 335, 449, 479, 632, 637, 657, 881, 1016, 1046, 1062, 1104, 1116, 1123, 1126, 1226, 1256, 1275, 1285, 1303, 1322, 1337, 1350, 1351, 1356, 1358, 1398, 1421, 1424, 1442, and 1446). The Biken M gene differed from the Edmonston M gene at 65 positions but from the Nagahata M gene at only 36 positions (Fig. 2, asterisks; Table 1). Therefore, Biken strain SSPE virus is more closely related to Nagahata strain measles virus than to any other known acute measles virus strain.

Possible evolutionary relationship between Nagahata strain acute measles virus and Biken strain SSPE virus. Biken strain was isolated in 1974 from a 6-year-old patient with SSPE in Ikeda City, Osaka Prefecture, Japan, near where Nagahata strain was isolated 3 years earlier (37). The present data strongly suggest that Biken strain SSPE virus was derived from a progenitor closely related to Nagahata strain measles virus. Interestingly, many of the nucleotides in the Nagahata M genes which differed from the consensus M sequence of previously studied acute measles virus strains were also found in several other SSPE virus strains isolated in diverse locations. Specifically, 20 of the 36 changes shared between the Nagahata and Biken M genes (nucleotides 77, 290, 297, 479, 632, 657, 881, 1046, 1116, 1123, 1226, 1256, 1275, 1285, 1303, 1337, 1350, 1358, 1421, and 1446) were identical to the corresponding changes in the M genes of the Niigata and Yamagata SSPE virus strains, which were isolated in different parts of Japan (18, 19, 23, 41) (Fig. 2, Ni and Ym). The majority of these common changes (except nucleotide 1446) also matched the changes in the M gene of IP-3 strain SSPE virus (Fig. 2, Ip), which was isolated in the United States (11, 13). Furthermore, many of these common nucleotides were also present in the M genes of the M-F and K strain SSPE viruses from Europe (3, 16, 26).

Table 1 shows the compositions of the nucleotide differences in four SSPE viral M genes compared with either the consensus M sequence of previously known acute measles virus strains or the Nagahata M gene. Using the Nagahata M gene as a reference standard, the numbers of nucleotide differences in both the Biken and IP-3 M genes were greatly reduced (Table 1). The total nucleotide differences in the M genes of the Niigata and Yamagata strains remained roughly the same regardless of whether they were compared with the Nagahata or consensus M sequence (Table 1). This suggests that Nagahata virus is more closely related to Biken and IP-3 strain SSPE viruses and that Niigata and Yamagata strains are derived from progenitors distinct from currently known acute measles virus, including Nagahata and Edmonston strains.

Role of biased hypermutation in evolution of Biken strain SSPE virus. Assuming that Biken strain SSPE virus was derived from an ancestral virus similar to Nagahata strain acute measles virus, the nucleotide differences between the Biken and Nagahata M genes could now be considered as mutations. Twenty-nine of the mutations (80%) in the Biken M gene were U-to-C transitions (Table 1). These unusual nucleotide changes, called biased hypermutation, have been observed in a number of measles virus strains that infect the CNS (14, 41) and are postulated to be a manifestation of a cellular ribonucleotide deamination activity (5, 6, 36, 41). Excluding the U-to-C changes, the Nagahata and Biken M genes became nearly identical (Fig. 2; Table 1). In total, 25 mutations (of all types) occurred in the Biken M protein-coding region, and 11 were in the long 3′-untranslated region of the M gene (Fig. 2, asterisks). Sixteen of the mutations were predicted to cause amino acid changes in the Biken M protein (Fig. 2, asterisks in triangles). Except for nucleotide

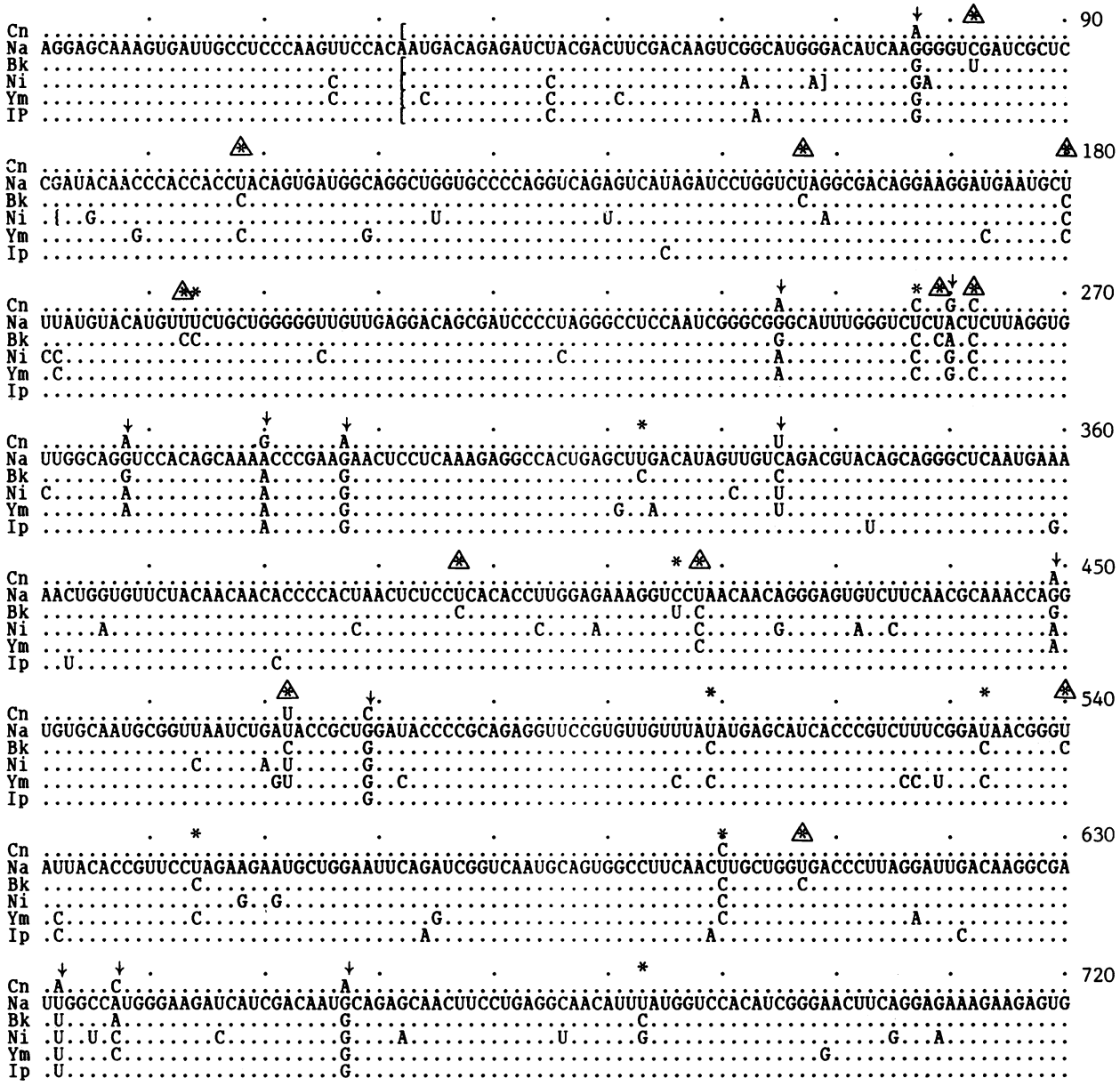

FIG. 2. Comparison among M genes of Nagahata virus and other acute and chronic measles virus strains. The Nagahata M gene (Na) was compared with the consensus M sequence (Cn) of other known acute measles virus strains (7, 14, 17, 42) and the M genes of Biken (Bk), Niigata (Ni), Yamagata (Ym), and IP-3 (Ip) SSPE viruses (1, 13, 19, 41). Open brackets ([) at position 33 represent the translational start sites. Close brackets (]) between positions 1040 and 1085 denote the predicted translation termination sites for the various M genes. The AUG codon in the Yamagata M gene is mutated to ACG, but translation initiates at low efficiency at this position (2). Translation of the Niigata M gene prematurely terminates at position 66 and reinitiates at position 93 (2). The aberrant start and stop codons for the cryptic M protein-coding frames in the Niigata and Yamagata M genes are shown by { and }, respectively. Nucleotides shown as dots are identical to the Nagahata sequence. Asterisks indicate nucleotides which differ between the Nagahata and Biken M genes. Missense mutations leading to amino acid substitutions are indicated by asterisks in triangles. Arrows indicate nucleotides which are in common between the Nagahata and Biken M genes but differ from the consensus M sequence. Nucleotide 1111, shown as a lowercase letter in Ym, and the number of Cs between nucleotides 1071 and 1078 in Na are ambiguous.

82, all these missense mutations turned out to be U-to-C transitions.

Therefore, biased hypermutation plays a central role in altering the structure of the Biken virus M protein. Notably, the M genes of the Niigata and Yamagata SSPE virus strains also contain a large number of U-to-C transitions (Table 1). The significance of these mutations in the evolution of SSPE virus will be discussed below (see Discussion).

Effects of mutations on M protein structure. To learn how the mutations affect the conformation of the measles virus M protein, we examined the M proteins of two acute measles virus strains (Nagahata and Edmonston) and three SSPE

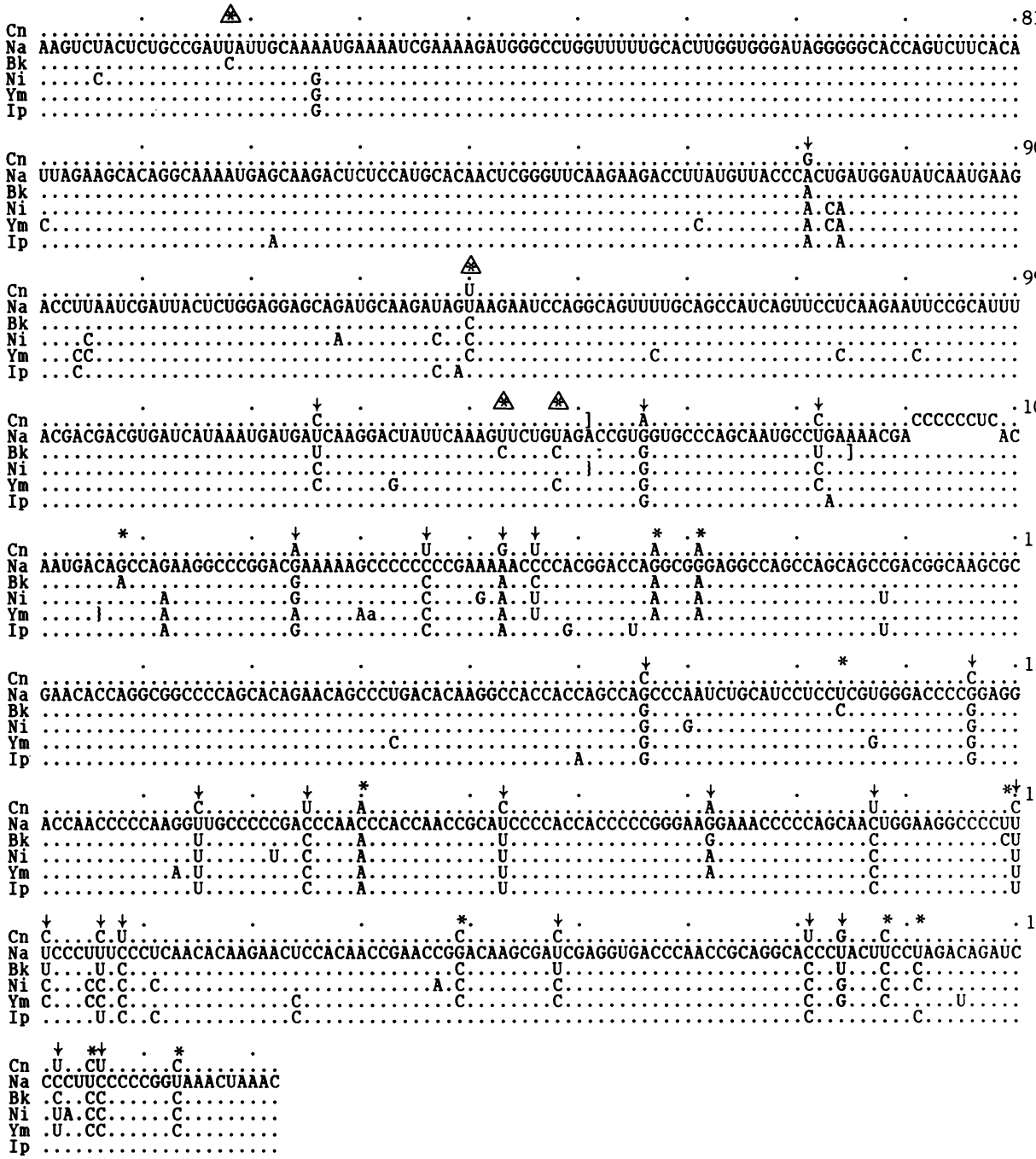

FIG. 2—*Continued*

virus strains (Biken, Niigata, and Yamagata) with antisera made against either the native or denatured form of M protein. Mutations interrupted the M protein coding frame in Niigata strain SSPE virus and destroyed the translational start codon for M protein in Yamagata strain SSPE virus (19, 41). However, these mutant M genes produced low levels of aberrant cryptic M proteins by use of alternative translational start sites (2). To permit comparative studies on the M proteins of these SSPE virus strains, we increased the synthesis of the cryptic M proteins by removing the upstream open reading frame from the Niigata M gene and restoring the translational start codon in the Yamagata M gene (2). Radiolabeled M proteins were synthesized in vitro from the repaired Niigata (NMd85) and Yamagata (YM2A) M genes, as well as the M genes of Biken (BM1 and BM2 [1]), Edmonston (EM1 [42]), and Nagahata (NagM) strains. Equal amounts of the M proteins synthesized in vitro (Fig. 3, T) were immunoprecipitated.

The GM antiserum against the native proteins of Nagahata strain immunoprecipitated the M proteins of both the Edmonston and Nagahata strains (Fig. 3, GM, lane a; Fig. 1, lanes c and d). This antiserum did not recognize the M

TABLE 1. Genetic changes in SSPE virus M genes compared with consensus[a] and Nagahata[b]

Change	Biken[c] vs:		Niigata[d] vs:		Yamagata[e] vs:		IP-3[f] vs.	
	Consensus (%)	Nagahata (%)	Consensus (%)	Nagahata (%)	Consensus (%)	Nagahata (%)	Consensus (%)	Nagahata (%)
A to G	9 (13.8)	0 (0.0)	13 (17.6)	9 (11.7)	8 (11.6)	5 (6.7)	8 (17.4)	2 (7.7)
A to C	0 (0.0)	0 (0.0)	0 (0.0)	1 (1.3)	0 (0.0)	1 (1.4)	1 (2.2)	1 (3.8)
A to U	1 (1.5)	0 (0.0)	2 (2.7)	1 (1.3)	1 (1.4)	0 (0.0)	1 (2.2)	0 (0.0)
G to A	7 (10.8)	3 (8.3)	18 (24.3)	19 (24.7)	10 (14.5)	13 (17.6)	10 (21.7)	8 (30.8)
G to C	0 (0.0)	1 (2.8)	0 (0.0)	1 (1.3)	0 (0.0)	1 (1.4)	1 (2.2)	1 (3.8)
G to U	2 (3.1)	0 (0.0)	1 (1.4)	1 (1.3)	0 (0.0)	0 (0.0)	0 (0.0)	0 (0.0)
C to A	1 (1.5)	1 (2.8)	2 (2.7)	3 (3.9)	1 (1.4)	2 (2.7)	2 (4.3)	2 (7.7)
C to G	3 (4.6)	0 (0.0)	3 (4.1)	0 (0.0)	7 (10.1)	2 (2.7)	3 (6.5)	0 (0.0)
C to U	9 (13.8)	2 (5.6)	5 (6.8)	7 (9.1)	5 (7.2)	8 (10.8)	8 (17.4)	4 (15.4)
U to A	0 (0.0)	0 (0.0)	0 (0.0)	0 (0.0)	0 (0.0)	0 (0.0)	0 (0.0)	0 (0.0)
U to G	0 (0.0)	0 (0.0)	1 (1.4)	2 (2.6)	1 (1.4)	2 (2.7)	0 (0.0)	0 (0.0)
U to C	33 (50.8)	29 (80.6)	29 (39.2)	33 (42.9)	36 (52.5)	40 (54.5)	12 (26.1)	8 (30.8)

[a] Based on M sequences of Edmonston, CAM, and Hu-2 strains of acute measles virus (7, 14, 17, 42).
[b] See Fig. 2.
[c] Reference 1.
[d] References 2 and 19.
[e] Reference 41.
[f] Reference 13.

proteins of the Biken, Niigata, and Yamagata SSPE virus strains (Fig. 3, GM, lanes b to e). On the other hand, the M-BC antiserum against the denatured Edmonston M protein not only recognized the M proteins of the Edmonston and Nagahata strains (Fig. 3, BC, lanes a, f, and g) but also reacted with the M proteins of all three SSPE viruses with varying efficiency (Fig. 3, BC, lanes h and b to e). The M-BBC antiserum made against the denatured Biken M protein cross-reacted strongly with the M proteins of all three SSPE viruses (Fig. 3, BBC, lanes b, c, d, e, and h), yet failed to react efficiently with the M proteins of the Edmonston and Nagahata strains (Fig. 3, BBC, lanes a, f, and g).

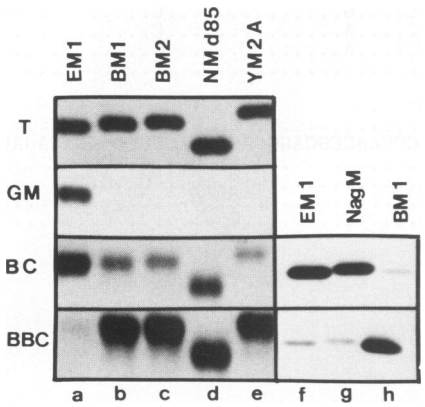

FIG. 3. Distinct antigenic determinants in M proteins of acute measles virus and SSPE virus strains. RNA was transcribed in vitro from the M cDNAs of the Edmonston (EM1), Nagahata (NagM), or Biken (BM1 and BM2 clones [1]) strains or from the modified M genes of the Niigata and Yamagata strains (NMd285 and YM2A, respectively), which produced high levels of the cryptic M proteins (2). Equal amounts of [^{35}S]methionine-labeled M proteins were translated from the RNA in reticulocyte lysates and immunoprecipitated with the GM antiserum against total nondenatured Nagahata viral proteins (GM), the M-BC antiserum against the denatured M protein of the Edmonston strain (BC), or the M-BBC antiserum against the denatured M protein of the Biken strain (BBC) (1, 2). The immunoprecipitated proteins were analyzed by SDS-10% PAGE in parallel with the total nonimmunoprecipitated in vitro translation products (T).

These results indicate that the M proteins of Nagahata and Edmonston strain acute measles viruses possess conformation-specific epitopes which are efficiently recognized by antibodies against the native M protein of acute measles virus. These conformation-specific epitopes are lacking in the M proteins of the Biken, Niigata, and Yamagata strain SSPE viruses, which only react with sequence-specific antibodies against the denatured M protein of either an acute measles virus or an SSPE virus. Finally, the fact that the M-BBC antiserum which cross-reacts with the M proteins of three SSPE virus strains does not react efficiently with the Nagahata or Edmonston M protein suggests that the sequence-specific epitopes recognized by this antiserum are inaccessible in the properly folded M protein of acute measles virus.

DISCUSSION

Measles virus strains that cause chronic CNS infections often display a variety of biological and biochemical abnormalities. These abnormalities typically affect expression, stability, or function of viral gene products involved in virion maturation, such as M (1, 2, 4, 12, 13, 15, 16, 28, 33) or F or H (4, 28) protein. The viral defects are believed to be due to genetic changes in the viral genomes. Indeed, the nucleotide sequences of a number of measles virus strains that cause chronic CNS infections diverge considerably from those that cause acute measles (for a review, see reference 16). However, since the direct progenitors of the chronic measles virus strains have heretofore not been isolated, it has been difficult to ascertain the significance of the sequence disparity. Specifically, if chronic measles virus strains are derived from progenitors similar to currently known acute measles virus strains, the genetic differences between the chronic and acute measles virus strains could represent mutations acquired during chronic infection. Alternatively, if the progenitors of chronic measles virus strains are distinct from the known acute measles virus strains, then some of the changes might be irrelevant to chronic infection and might merely

reflect genetic variations between the true progenitor and the reference viruses.

This study identifies an acute measles virus, Nagahata strain, with an M gene more closely related to the M gene of Biken strain SSPE virus than to the M genes of all the previously characterized isolates of acute measles virus. The sequence similarity and the time and location of isolation suggest a likely evolutionary relationship between the Nagahata and Biken strains. Comparison of the Nagahata and Biken strains with other acute and chronic strains of measles virus provides a number of useful insights into the evolutionary process of SSPE virus.

First, the data reveal a high degree of conservation in the M proteins of acute measles virus strains. The M genes of Nagahata and Edmonston strain measles viruses differ by 46 nucleotides, yet these nucleotide differences lead to only four amino acid substitutions in the predicted M proteins (Pro-77 to Leu-77, Lys-89 to Glu-89, Pro-202 to His-202, and Thr-209 to Ala-209; corresponding to nucleotides 260, 290, 632, and 657, respectively, Fig. 2). Considering that the Nagahata and Edmonston strains were isolated more than 20 years apart from different continents and that the Edmonston strain has been passaged in vitro in various laboratories for over 30 years, the remarkable conservation of M protein suggests that the M protein of acute measles virus must meet very stringent structural requirements and that the functional protein structure confers the conformation-specific epitopes that are recognized by antibodies against the native M protein.

Second, the M protein of the Biken strain and the cryptic M proteins of the Niigata and Yamagata strain SSPE viruses do not react with antibodies against the native M protein of acute measles virus, yet these SSPE viral M proteins are recognized by antibodies against the denatured M protein of either acute measles virus or SSPE virus (Fig. 3). This suggests that the SSPE viral M proteins fail to fold into a proper tertiary structure, thereby exposing sequence-specific epitopes that are recognized by antibodies against the denatured M protein. In light of the strict structural requirement for M protein function, conformational changes in the M protein would most likely abolish the protein function and contribute a nonproductive mode of virus replication. The present findings do not exclude the possibility that defects in other viral proteins besides M could also cause nonproductive infection.

Third, the genetic changes in measles virus strains fall into two categories. Most of the nucleotide differences between the M genes of the Nagahata and Edmonston strain acute measles viruses appear to be random and can be explained by errors in viral RNA replication (Fig. 2, arrows). In contrast, 80% of the differences between the Biken and Nagahata M genes are U-to-C transitions, which cannot be easily accounted for by random polymerase errors (Table 1). In other words, most of the genetic variations between the M genes of acute measles virus strains might be due to random polymerase errors, whereas the major distinction between the M genes of the Biken strain SSPE virus and the putative progenitor is ascribable to biased hypermutation.

How does biased hypermutation affect the M gene function? Since translational termination codons do not contain C residues, U-to-C transitions by themselves would not introduce new termination codons. However, U-to-C transitions could drastically alter the M protein sequence. In fact, 15 of the 16 missense mutations in the Biken M gene are U-to-C transitions, causing 14 predicted amino acid substitutions and 8 additions (Fig. 2). Therefore, biased hypermutation is almost solely responsible for the marked structural changes in the M protein of Biken strain SSPE virus (1). Besides causing an amino acid substitution, a U-to-C transition could also destroy a translational start AUG codon. Notably, such a mutation abolishes the translational function of the M gene of Yamagata strain SSPE virus (Fig. 2, Ym) (2, 41). In other SSPE virus strains (e.g., the Niigata strain), the critical mutations that destroy the M gene function might not be U-to-C transitions (Fig. 2, Ni) (2). However, the numerous U-to-C changes could prevent reversion of the mutant M genes.

Biased hypermutation is thought to be caused by a cellular RNA-modifying activity (5, 6, 36, 41). However, the known cellular RNA-modifying enzyme is ubiquitous (5, 36). How can this be reconciled with the rarity of hypermutated viral RNA sequences? Since the RNA-modifying activity is confined to the cellular nucleus, perhaps the cytoplasmic site of replication spares the genomes of most RNA viruses from modification. Also, the known cellular RNA-modifying activity prefers double-stranded RNA as a substrate. Many viral genomes, particularly those of negative-strand RNA viruses, are encapsidated by viral proteins and might not be an ideal target for the RNA-modifying activity. Furthermore, hypermutated viral genomes defective in a replicative function might be quickly eliminated in a normal acute virus infection. For the same reason, hypermutation might be tolerated in viral sequences that are not under strong selective pressure. It is noteworthy that the long 3′-untranslated region of the M gene and the 5′-untranslated region of the F gene of measles virus both have unusually high C:U ratios (41), and many of the U versus C differences between the Nagahata and Edmonston M genes occur in the 3′-untranslated region (Fig. 2, Na and Cn).

Finally, the sequence divergence between Nagahata and previously characterized strains of acute measles virus is of interest. The derivatives of Edmonston strain isolated from the United States (20, 21), CAM strain from Japan (25, 35, 38, 39), and Hu-2 strain from Ireland (29) all have similar nucleotide sequences. So far, only one measles virus (Halle strain) isolated from a patient with SSPE has Edmonston-like nucleotide sequences (9, 10, 16, 22). However, the Halle strain was not isolated from the brain but from a lymph node of the patient (24) and strictly speaking is not a typical SSPE virus. By contrast, Nagahata-like nucleotide sequences are found in Biken, Niigata, and Yamagata strains from Japan (18, 23, 37), the IP-3 strain from the United States (11), and MF and K strains from Europe (3, 26). At first glance, these observations suggest that measles virus strains related to the Nagahata strain are more prone to cause CNS infections than relatives of the Edmonston strain. However, the apparent relatedness between Nagahata and SSPE virus strains might reflect the continuous changes in measles virus sequences with time. Among the previously studied acute measles virus strains, the Edmonston and CAM strains were isolated in the 1950s and the Hu-2 strain was isolated in 1971 from a patient after immunization with a Schwarz strain vaccine (29), which was derived from the Edmonston strain. Thus, the Nagahata strain might be the most recent isolate among the acute measles virus strains characterized and therefore might be a closer relative of the SSPE virus strains isolated in recent decades. Studying more measles virus isolates from different outbreaks might be useful for further understanding the evolutionary pattern of measles virus.

In summary, the present study identifies the likely origin of Biken strain SSPE virus, shows that biased hypermutation is largely responsible for the Biken M protein defect,

and suggests that biased hypermutation plays an important role in the evolution of SSPE virus.

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REFERENCES

1. **Ayata, M., A. Hirano, and T. C. Wong.** 1989. Structural defect linked to nonrandom mutations in the matrix gene of Biken strain subacute sclerosing panencephalitis virus defined by cDNA cloning and expression of chimeric genes. J. Virol. **63**:1162–1173.
2. **Ayata, M., A. Hirano, and T. C. Wong.** 1991. Altered translation of the matrix genes in Niigata and Yamagata neurovirulent measles virus strains. Virology **180**:166–174.
3. **Baczko, K., M. J. Carter, M. Billeter, and V. ter Meulen.** 1984. Measles virus gene expression in subacute sclerosing panencephalitis. Virus Res. **1**:585–595.
4. **Baczko, K., U. G. Liebert, M. Billeter, R. Cattaneo, H. Budka, and V. ter Meulen.** 1986. Expression of defective measles virus genes in brain tissues of patients with subacute sclerosing panencephalitis. J. Virol. **59**:472–478.
5. **Bass, B. L., and H. Weintraub.** 1988. An unwinding activity that covalently modifies its double-stranded RNA substrate. Cell **55**:1089–1098.
6. **Bass, B. L., H. Weintraub, R. Cattaneo, and M. A. Billeter.** 1989. Biased hypermutation of viral RNA genomes could be due to unwinding/modification of double-stranded RNA. Cell **56**: 331.
7. **Bellini, W. J., G. Englund, C. D. Richardson, S. Rozenblatt, and R. A. Lazzarini.** 1986. Matrix genes of measles virus and canine distemper virus: cloning, nucleotide sequences, and deduced amino acid sequences. J. Virol. **58**:408–416.
8. **Billeter, M. A., R. Cattaneo, A. Schmid, D. Eschle, K. Kaelin, G. Rebmann, S. A. Udem, R. D. Sheppard, K. Baczko, U. G. Liebert, S. Schneider-Schaulies, U. Brinckmann, and V. ter Meulen.** 1989. Host and viral features in persistent measles virus infection of the brain, p. 356–366. *In* B. W. J. Mahy and D. Kolakofsky (ed.), Genetics and pathogenicity of negative strand viruses. Elsevier/North-Holland Publishing Co., Amsterdam.
9. **Buckland, R., C. Gerald, D. Barker, and F. Wild.** 1987. Fusion glycoprotein of measles virus: nucleotide sequence of the gene and comparison with other paramyxoviruses. J. Gen. Virol. **68**:1695–1703.
10. **Buckland, R., C. Gerald, D. Barker, and F. Wild.** 1988. Cloning and sequencing of the nucleoprotein gene of measles virus (Halle strain). Nucleic Acids Res. **16**:11821.
11. **Burnstein, T., L. B. Jacobsen, W. Zeman, and T. T. Chen.** 1974. Persistent infection of BSC-1 cells by defective measles virus derived from subacute sclerosing panencephalitis. Infect. Immun. **10**:1378–1382.
12. **Carter, M. J., M. M. Willcocks, and V. ter Meulen.** 1983. Defective translation of measles virus matrix protein in a subacute sclerosing panencephalitis cell line. Nature (London) **305**:153–155.
13. **Cattaneo, R., A. Schmid, M. A. Billeter, R. D. Sheppard, and S. A. Udem.** 1988. Multiple viral mutations rather than host factors cause defective measles virus gene expression in a subacute sclerosing panencephalitis cell line. J. Virol. **62**:1388–1397.
14. **Cattaneo, R., A. Schmid, D. Eschle, K. Baczko, V. ter Meulen, and M. A. Billeter.** 1988. Biased hypermutation and other genetic changes in defective measles virus in human brain infections. Cell **55**:255–265.
15. **Cattaneo, R., A. Schmid, G. Rebmann, K. Baczko, V. ter Meulen, W. J. Bellini, S. Rozenblatt, and M. A. Billeter.** 1986. Accumulated measles virus mutations in a case of subacute sclerosing panencephalitis: interrupted matrix protein reading frame and transcription alteration. Virology **154**:97–107.
16. **Cattaneo, R., A. Schmid, P. Spielhofer, K. Kaelin, K. Baczko, V. ter Meulen, J. Pardowitz, S. Flanagan, B. K. Rima, S. Udem, and M. A. Billeter.** 1989. Mutated and hypermutated genes of persistent measles viruses which caused lethal human brain diseases. Virology **173**:415–425.
17. **Curran, M. D., and B. K. Rima.** 1988. Nucleotide sequence of the gene encoding the matrix protein of a recent measles virus isolate. J. Gen. Virol. **69**:2407–2411.
18. **Doi, Y., T. Sampe, M. Nakajima, S. Okawa, T. Kato, H. Ito, T. Sato, K. Oguchi, T. Kumanishi, and T. Tsubaki.** 1972. Properties of a cytopathic agent isolated from a patient with subacute sclerosing panencephalitis in Japan. Jpn. J. Med. Sci. Biol. **25**:321–333.
19. **Enami, M., T. A. Sato, and A. Sugiura.** 1989. Matrix protein of cell-associated subacute sclerosing panencephalitis viruses. J. Gen. Virol. **70**:2191–2196.
20. **Enders, J. F., S. L. Katz, and A. Holloway.** 1962. Development of attentuated measles virus vaccines. A summary of recent investigation. Am. J. Dis. Child **103**:335–340.
21. **Enders, J. F., and T. C. Peebles.** 1954. Propagation in tissue cultures of cytopathic agents from patients with measles. Proc. Soc. Exp. Biol. Med. **86**:277–286.
22. **Gerald, C., R. Buckland, R. Barker, G. Freeman, and T. F. Wild.** 1986. Measles virus haemagglutinin gene: cloning, complete nucleotide sequence analysis and expression in COS cells. J. Gen. Virol. **67**:2695–2703.

22a. **Hirano, A.** Unpublished data.

23. **Homma, M., M. Tashiro, H. Konno, Y. Ohara, M. Hino, and S. Takase.** 1982. Isolation and characterization of subacute sclerosing panencephalitis virus (Yamagata-1 strain) from a brain autopsy. Microbiol. Immunol. **26**:1195–1202.
24. **Horta-Barbosa, L., R. Hamilton, B. Wittig, D. A. Fuccillo, J. L. Sever, and M. L. Vernon.** 1971. Subacute sclerosing panencephalitis: isolation of suppressed measles virus from lymph node biopsies. Science **173**:840–841.
25. **Kobune, K., F. Kobune, K. Yamanouchi, K. Nagashima, Y. Yoshikawa, and M. Hayami.** 1983. Neurovirulence of rat brain-adapted measles virus. Jpn. J. Exp. Med. **53**:177–180.
26. **Kratzsch, V., W. W. Hall, K. Nagashima, and V. ter Meulen.** 1977. Biological and biochemical characterization of a latent subacute sclerosing panencephalitis (SSPE) virus infection in tissue culture. J. Med. Virol. **1**:139–154.
27. **Kristensson, K., and E. Norrby.** 1986. Persistence of RNA viruses in the central nervous system. Annu. Rev. Microbiol. **40**:159–184.
28. **Liebert, U. G., K. Baczko, H. Budka, and V. ter Meulen.** 1986. Restricted expression of measles virus proteins in brains from cases of subacute sclerosing panencephalitis. J. Gen. Virol. **67**:2435–2444.
29. **Mawhinney, H., I. V. Allen, J. M. Beare, J. M. Bridges, J. H. Connoly, M. Haire, N. C. Nevin, D. W. Neill, and J. R. Hobbs.** 1971. Dysgammaglobulinaemia complicated by disseminated measles. Br. Med. J. **2**:380–381.
30. **Norrby, E., and M. N. Oxman.** 1990. Measles virus, p. 1013–1044. *In* B. N. Fields (ed.), Virology, vol. 1. Raven Press, New York.
31. **Roux, L., P. Beffy, and A. Portner.** 1984. Restriction of cell surface expression of Sendai virus hemagglutinin-neuraminidase glycoprotein correlates with its higher instability in persistently and standard plus defective interfering virus infected BHK-21 cells. Virology **138**:118–128.
32. **Sanger, F., S. Nicklen, and A. R. Coulson.** 1977. DNA sequencing with chain-terminating inhibitors. Proc. Natl. Acad. Sci. USA **74**:5463–5467.
33. **Sheppard, R. D., C. S. Raine, M. B. Bornstein, and S. A. Udem.** 1986. Rapid degradation restricts measles virus matrix protein expression in a subacute sclerosing panencephalitis cell line. Proc. Natl. Acad. Sci. USA **83**:7913–7917.
34. **ter Meulen, V., J. R. Stephenson, and H. W. Kreth.** 1983. Subacute sclerosing panencephalitis. Compr. Virol. **18**:105–185.
35. **Toyoshima, K., M. Takahashi, S. Hata, N. Kunita, and Y. Okuno.** 1959. Virological studies on measles virus. I. Isolation of measles virus using FL cells and immunological properties of the isolated agents. Biken J. **2**:305–312.
36. **Wagner, R. W., J. E. Smith, B. S. Cooperman, and K.**

Nishikura. 1989. A double-stranded RNA unwinding activity introduces structural alterations by means of adenosine to inosine conversions in mammalian cells and Xenopus eggs. Proc. Natl. Acad. Sci. USA **86**:2647–2651.

37. **Ueda, S., Y. Okuno, Y. Okuno, Y. Hamamoto, and H. Ohya.** 1975. Subacute sclerosing panencephalitis (SSPE): isolation of a defective variant of measles virus from brain obtained at autopsy. Biken J. **18**:113–122.
38. **Ueda, S., M. Takahashi, T. Kurimura, Y. Minekawa, N. Suzuki, K. Yamanishi, K. Baba, Y. Okuno, T. Sasada, K. Onishi, T. Konobe, K. Takaku, Y. Yamada, T. Tanami, F. Kusano, Y. Hayakawa, and T. Kurose.** 1972. Development of extremely attenuated live measles virus vaccine (CAM-EX). Biken J. **15**:173–177.
39. **Ueda, S., M. Takahashi, Y. Minekawa, T. Ogino, N. Suzuki, K. Yamanishi, K. Baba, Y. Okuno, T. Konobe, T. Sasada, and K. Takaku.** 1970. Studies on further attenuated live measles vaccine. I. Adaptation of measles virus to the chorioallantoic membrane of chick embryo and clinical tests on the strain. Biken J. **13**:111–116.
40. **Wechsler, S. L., and H. Cody Meissner.** 1982. Measles and SSPE viruses: similarities and differences. Prog. Med. Virol. **28**:65–95.
41. **Wong, T. C., M. Ayata, A. Hirano, Y. Yoshikawa, H. Tsuruoka, and K. Yamanouchi.** 1989. Generalized and localized biased hypermutation affecting the matrix gene of a measles virus strain that causes subacute sclerosing panencephalitis. J. Virol. **63**:5464–5468.
42. **Wong, T. C., G. Wipf, and A. Hirano.** 1987. The measles virus matrix gene and gene product defined by in vitro and in vivo expression. Virology **157**:497–508.