

Expression of Defective Measles Virus Genes in Brain Tissues of Patients with Subacute Sclerosing Panencephalitis

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The persistence of measles virus in selected areas of the brains of four patients with subacute sclerosing panencephalitis (SSPE) was characterized by immunohistological and biochemical techniques. The five measles virus structural proteins were never simultaneously detectable in any of the brain sections. Nucleocapsid proteins and phosphoproteins were found in every diseased brain area, whereas hemagglutinin protein was detected in two cases, fusion protein was detected in three cases, and matrix protein was detected in only one case. Also, it could be shown that the amounts of measles virus RNA in the brains differed from patient to patient and in the different regions investigated. In all patients, plus-strand RNAs specific for these five viral genes could be detected. However, the amounts of fusion and hemagglutinin mRNAs were low compared with the amounts in lytically infected cells. The presence of particular measles virus RNAs in SSPE-infected brains did not always correlate with mRNA activity. In *in vitro* translations, the matrix protein was produced in only one case, and the hemagglutinin protein was produced in none. These results indicate that measles virus persistence in SSPE is correlated with different defects of several genes which probably prevent assembly of viral particles in SSPE-infected brain tissue.

Subacute sclerosing panencephalitis (SSPE) in humans is a chronic inflammatory central nervous system disease process based on a persistent measles virus infection (1). In infected brain cells, measles virus nucleocapsids are detectable in the absence of budding virus particles and the formation of giant cells. This observation together with the failure to isolate directly infectious virus from brain tissue suggests impaired measles virus replication in brain cells which has been attributed to the expression of a single defective measles virus gene (31). This conclusion is based on the absence of a humoral immune response to measles virus matrix (M) protein in SSPE patients (14) and the lack of M protein in SSPE-infected brain tissue (13) as well as on biochemical data obtained from studies of persistently infected cells derived from SSPE-infected brain tissue which indicate that a restriction of measles virus M protein expression is a consequence of either a block in translation (10) or the failure of the synthesized protein to accumulate (28). Similar observations have been made directly on SSPE-infected brain tissue. Northern blot analysis in combination with *in vitro* translation of extracted measles virus RNA from brain tissue (4) or hybridization tomography together with immunocytochemical staining of SSPE-infected brain sections (12) reveals the presence of mRNA for the M protein without M protein synthesis. However, measles virus M protein has recently been found in SSPE-infected brain sections by using monoclonal antibodies (21). In these cases, a restriction of the M mRNA either does not occur or is less effective, suggesting that this particular viral gene may not alone determine the persistence of measles virus. To obtain more detailed information on the presence of measles virus-specific RNA species and the expression of mRNAs in SSPE-infected brain tissue and to evaluate the results in an appropriate context, it was necessary to study SSPE cases in

which information about the presence of viral structural proteins in brain areas was available. In the present study, RNAs obtained from different areas of the brains of four such defined SSPE cases were characterized by both Northern blot hybridization with cDNA clones from five measles virus genes and by the ability of the mRNAs to direct the *in vitro* synthesis of viral proteins. The data obtained indicate a great variability in the pattern of mRNA accumulation and an altered expression of defective genes not only for M protein but also for the genes encoding the envelope proteins of measles virus. Thus, the defects in the replication of measles virus seen in the SSPE-infected brain pertain to several viral genes.

MATERIALS AND METHODS

Patient 1. In a 9-year-old child who died after only a 3-month course of SSPE, hyperimmune responses to measles virus antigens, except for M protein, were found in cerebrospinal fluid (CSF) and serum. Hemagglutination-inhibiting antibodies to measles virus were seen in serum but not in CSF. Neuropathologically, the brain revealed the typical diffuse encephalitis of varying severity in both the grey and white matter with the characteristic Cowdry type A inclusion bodies.

Patient 2. In a 10-year-old child who died after a 6-month course of SSPE, hyperimmune responses to measles virus antigens except for M protein, were found in CSF and serum. Neuropathological findings consisted of perivascular lymphoplasmal cellular infiltrations, Cowdry type A inclusion bodies in neurons and oligodendrocytes, and reactive fibrillar astrocytes in the grey and white matter of the entire brain.

Patient 3. In a 14-year-old child who died after an 18-month course of SSPE, hyperimmune responses to measles virus antigens were found in CSF and serum. No antibodies to M protein were detectable. Neuropathological examina-

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tions revealed the characteristic Cowdry type A inclusion bodies in oligodendrocytes, astrocytes, and ganglion cells, together with lymphoplasmic cellular infiltrations predominantly in the frontal and temporal lobes.

Patient 4. A 16-year-old child who died 6 months after onset of SSPE had, like the other three patients, a typical humoral hyperimmune response to measles virus antigens with the exception of M protein. Neuropathological findings included meningitis, prominent perivascular infiltrations by lymphocytes and plasma cells of the brain, and focal destruction of ganglion cell layers and white matter. Cowdry type A inclusion bodies were only occasionally seen.

Tissue processing. Brain material from our patients was obtained within a few hours after death, immediately frozen, and stored at -70°C . Attempts to isolate infectious virus by cocultivation techniques from the brain tissues of patients 1, 3, and 4 failed. Such experiments were not carried out with the brain tissue of patient 2.

Immunofluorescence. Frozen brain sections ($8\ \mu\text{m}$ thick) were cut on a Cryostat, fixed in acetone (-20°C) for 10 min, and incubated in 10% normal horse serum in Tris-buffered saline (pH 7.4) for 30 min, followed by incubation with monoclonal antibodies against measles virus structural proteins. The antibodies were diluted 1:20 to 1:200 in Tris-buffered saline containing 5% normal horse serum for 60 min at room temperature. After a wash in buffer, sections were incubated for 60 min in fluorescein-labeled goat anti-mouse immunoglobulin G whole molecule (no. 62-6511; Conco Laboratories, Wiesbaden, Federal Republic of Germany) or $\text{F}(\text{ab}')_2$ fragments (no. 4350; Tago-Medac, Hamburg, Federal Republic of Germany) diluted 1:50 and 1:30, respectively. Sections were then washed again and mounted in glycerol. The specific fluorescence was evaluated in a Leitz fluorescence microscope.

Monoclonal antibodies. For this study, monoclonal antibodies against five structural components of measles virus, hemagglutinin (H), phospho- (P), fusion (F), nucleocapsid (N), and M proteins, were used. Monoclonal antibodies used from our own group included three monoclonal antibodies against H protein, two against N protein, two against F protein, and two against M protein which have been previously described (8, 9, 30). E. Norrby, Karolinska Institute, Stockholm, Sweden, kindly gave us three monoclonal antibodies against H protein, two against P protein, three against F protein, and three against M protein. The properties of these monoclonal antibodies have been previously described (20, 29). T. A. Sato, National Institute of Health, Tokyo, Japan, kindly gave us four monoclonal antibodies against F protein which have been described previously (27).

Virus and cells. Measles virus Edmonston was plaque purified three times in Vero cells. Stock virus was prepared by passaging the virus derived from individual plaques twice at low multiplicity (multiplicity of infection, 0.01) in Vero cells.

Extraction of RNA. Total cytoplasmic RNA was obtained as described previously (2, 4, 11). Briefly, Vero cells were infected at a multiplicity of infection of 0.01 with measles virus Edmonston and harvested when the cytopathic effect was almost complete. Cytoplasmic extracts were prepared and dissolved in guanidinium isothiocyanate with a Potter homogenizer. The cell homogenate was overlaid on a CsCl step gradient and centrifuged in an SW27 rotor at 20°C and 22,000 rpm overnight. The RNA pellet was dissolved in 7.5 M guanidinium chloride–40 mM acetic acid–25 mM sodium citrate (pH 7.0)–5 mM dithiothreitol, and 0.5 volume of ethanol was added. RNA was precipitated overnight at

-20°C , pelleted in an Eppendorf centrifuge, washed with 70% ethanol, dried, and dissolved in SEH buffer (100 mM NaCl, 1 mM EDTA, 10 mM HEPES [*N*-2-hydroxyethylpiperazine-*N'*-2-ethanesulfonic acid] [pH 7.0]). RNA was extracted from brain material (1 to 2 g) in the same way except that frozen brain samples were disrupted mechanically before RNA extraction. The average yield was $150\ \mu\text{g}$ of RNA from 1 g of brain tissue. Poly(A)⁺ RNAs were selected from total RNA by two cycles of oligo(dT)-cellulose chromatography.

In vitro translation. In vitro translation was carried out as previously described (4), by using a rabbit reticulocyte lysate (22) which was kindly provided by S. G. Siddell, Würzburg, Federal Republic of Germany.

Immunoprecipitation. Immunoprecipitations of in vitro translation products were carried out as follows. Proteins synthesized in vitro were diluted 10- to 20-fold in 1% Triton X-100–0.15 M NaCl–50 mM Tris (pH 7.2)–5 mM EDTA–0.1 mM phenylmethylsulfonyl fluoride buffer (19). For each 100 μl of diluted translation products, 2 μl of antiserum was added, and the dilution was incubated for 1 h at 4°C . The following antisera were used: sera or CSF from patients 1, 2, and 4; rabbit serum obtained after hyperimmunization with measles virus Edmonston; anti-H, -M, and -F polyclonal rabbit sera (kindly supplied by E. Norrby); a monoclonal anti-N serum; and six different monoclonal anti-F sera (three were kindly supplied by E. Norrby). The polyclonal sera were obtained in rabbits with viral proteins derived from sodium dodecyl sulfate-gel electrophoresis. Anti-P serum was prepared by immunization of rabbits with a synthetic peptide (kindly supplied by W. J. Bellini). After incubation, 20 μl of a slurry, consisting of a 1:1 (vol/vol) mixture of protein A-Sepharose CL-4B (Pharmacia Fine Chemicals, Piscataway, N.J.) and Machamer buffer (19) was added and incubated at 20°C with frequent shaking. After 20 min, the solid phase was washed three times with Machamer buffer. When monoclonal antibodies were used for immunoprecipitation, 50 mg of protein A-Sepharose CL-4B preswollen in RIPA buffer was incubated with 100 μl of rabbit anti-mouse immunoglobulins (Z109; DAKOPATTS GMBH, Hamburg, Federal Republic of Germany) for 45 min at 20°C and washed three times with Machamer buffer. Portions of 25 μl of 2 \times sample buffer (18) were added to the immune complexes between the antibodies and the bound in vitro translation products, and the solutions were incubated for 2 min at 100°C . The suspension was centrifuged in an Eppendorf centrifuge. The supernatant was electrophoresed in a 10 or 13% discontinuous polyacrylamide gel (acrylamide/bisacrylamide ratio, 1:77; 0.033% ammonium persulfate) containing 0.1% sodium dodecyl sulfate (18). After electrophoresis, gels were fixed in 7% acetic acid–50% methanol, fluorographed with Amplify (Amersham Corp.), dried, and exposed to preflashed Cronex 2 (E.I. du Pont de Nemours & Co., Inc., Wilmington, Del.) film.

Northern blots. Northern blots were carried out as described previously (4). Briefly, oligo(dT)-selected RNA samples were denatured with methyl mercury(II) hydroxide and electrophoresed in a 1% vertical agarose gel containing methyl mercury. After determination of the positions of 28S and 18S rRNAs, which were used as size markers, the RNA was transferred and bound to nitrocellulose. The filter was cut into strips, and each strip was hybridized with a different measles virus cDNA clone. Three of the clones used have been characterized previously. Clone 3' + N contains about 600 nucleotides from the 3' end of the genome (6); P contains approximately 400 nucleotides from the 3' end of the P

TABLE 1. Measles virus structural proteins detected in SSPE-infected brain tissue

| Protein | SSPE patient no.: | | | |
|---------|-------------------|---|---|---|
| | 1 | 2 | 3 | 4 |
| N | + | + | + | + |
| P | + | + | + | + |
| H | - | - | + | + |
| F | - | + | + | + |
| M | + | - | - | - |

message (5); and M contains about 450 nucleotides from the 3' end of the M message (25). In addition, the clone designated F-H contains 325 nucleotides from the F gene, 3 intercistronic nucleotides, and 88 nucleotides from the H gene (6; R. Cattaneo, unpublished data). The remaining clone contains approximately 900 nucleotides from the H gene, and it was designated H (6) because in *in vitro* translation experiments, this clone arrests the synthesis of a protein which reacts with the polyclonal anti-H serum kindly provided by E. Norrby (K. Baczko, unpublished data). The specific activity of the nick-translated inserts was 1×10^8 to 2×10^8 dpm/ μ g. After hybridization and washing, the strips were exposed to preflashed Cronex 2 film by using an intensifying screen.

RESULTS

Detection of measles virus structural proteins in SSPE-infected brain tissue. Cryostat sections were prepared from each brain area from which RNA was subsequently isolated and were analyzed for the presence of measles virus structural proteins by the immunofluorescence technique. For this assay, 24 different monoclonal antibodies against five structural proteins of measles virus were used, each reacting with a different epitope of the corresponding virus protein. This large panel of monoclonal antibodies was used to minimize the possibility of failing to recognize measles virus proteins as a result of antigenic changes during persistency. The N and P proteins were found in each of the four SSPE cases in every section recognized as infected (Table 1). However, the other virus proteins showed many differences. Patients 1 and 2 did not show H protein; F protein was absent in patient 1; and M protein was absent in patients 2, 3, and 4. Additionally, the number of brain cells exhibiting a positive fluorescence varied for each virus protein in the four patients, but the intensity of fluorescence for each virus protein was similar in all cases. Virus proteins were found in neurons, oligodendrocytes, and astrocytes. They were detected in the cytoplasm as well as in the nuclei of infected cells and often appeared as nuclear inclusions. In general, the distribution of the different virus structural proteins within infected brain cells corresponded to the findings of Norrby et al. (21).

Analysis of measles virus mRNAs in SSPE brain tissue by Northern blots. RNA was isolated from different parts of the brain of each patient, and poly(A)⁺ RNA was selected by two cycles of oligo(dT)-cellulose chromatography. Measles virus-specific mRNAs were identified by Northern blots by using cDNA clones from five measles virus genes. One representative Northern blot from each patient compared with blots from lytically infected Vero cells and from brain tissue used as a control is shown in Fig. 1. In blots from lytic infections (Fig. 1A), all the measles virus mRNAs investigated can be easily seen. In addition, weaker bands corre-

sponding to bicistronic transcripts are visible. As expected, hybridization with the F-H clone revealed two bands in the area of monocistronic transcripts, since this clone contains parts of the F and H genes. The analysis of the readthrough products confirms this designation (3; B. K. Rima et al., manuscript in preparation). No bands can be seen in blots from brain tissue used as a control. In the four patients, the quality and quantity of the measles virus transcripts were variable. N mRNA was always present. P and M mRNAs were present in patients 1, 2, and 4 (Fig. 1C to F). Patient 3 (Fig. 1E) showed a relatively high amount of the P-M readthrough product as well as some breakdown products.

The relative amounts of H and F mRNAs varied from

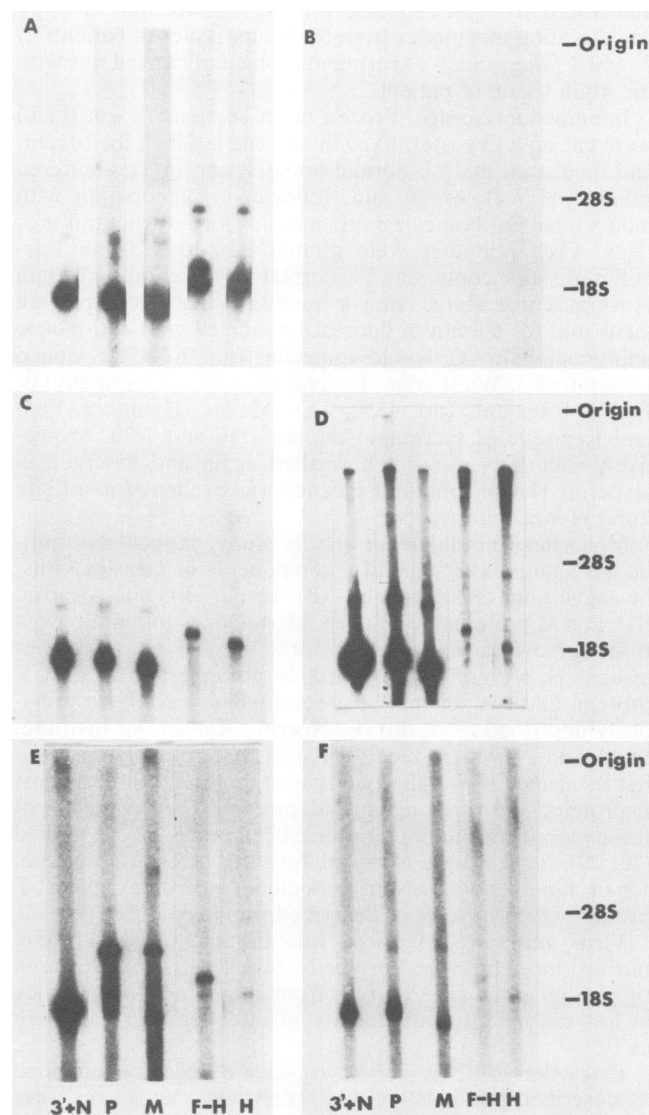


FIG. 1. Northern blot analysis of measles virus-specific RNAs. From 50 μ g of total RNA, poly(A)⁺ RNA was selected, applied to a gel slot (50 by 2 mm), electrophoresed, and transferred onto nitrocellulose. Strips of the resulting filter were hybridized to the 3' end of the genome and part of the N gene (3' + N), and to parts of the P, M, F and H (F-H), and H genes. 28S and 18S, the positions of rRNA. Panels show poly(A)⁺ RNA from lytically infected Vero cells (A), brain tissue used as a control (B), and brain tissue from patients 1 (C), 2 (D), 3 (E), and 4 (F). Exposure times: A, 2 days; B, 3 weeks; C, 1 day; D, 5 days; E, 1 day; F, 2 weeks.

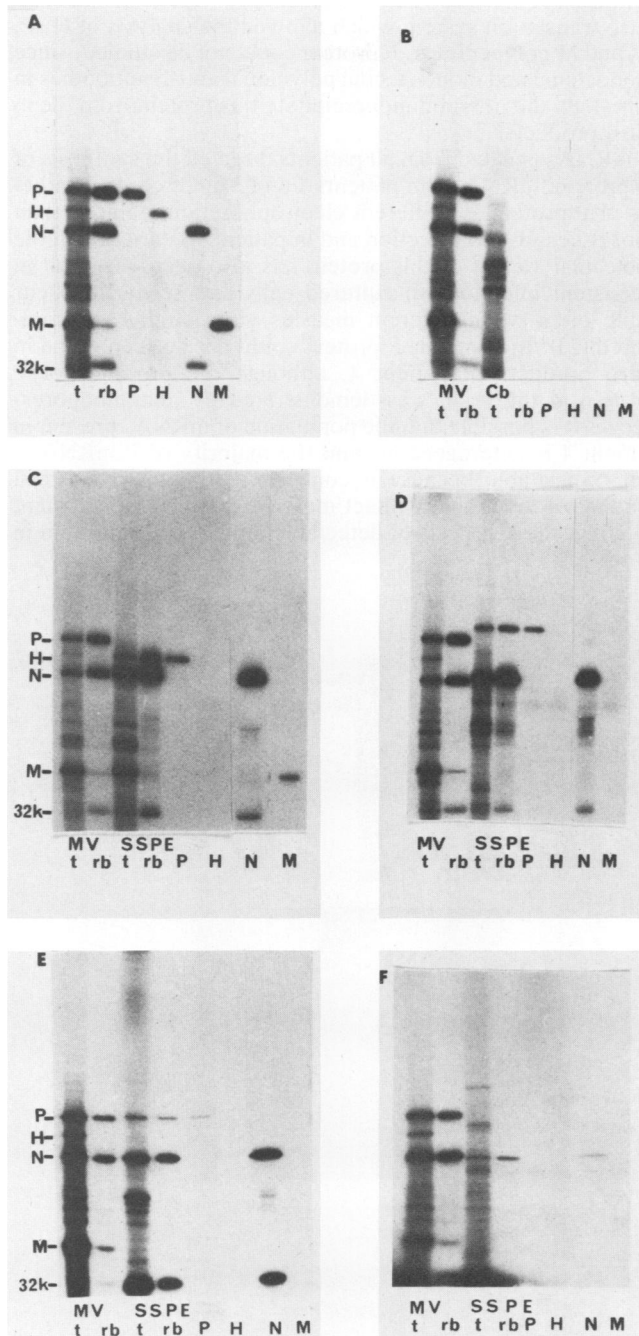


FIG. 2. In vitro translation of poly(A)⁺ RNAs derived from measles virus-infected tissue cultures and SSPE-infected brains. Poly(A)⁺ RNAs were translated in vitro by using a rabbit reticulocyte lysate system. Aliquots were analyzed either directly on a 10% sodium dodecyl sulfate-polyacrylamide gel or immunoprecipitated first with different antisera against measles virus: t, Total translate; rb, immunoprecipitation with rabbit hyperimmune serum; P, polyclonal anti-P protein; H, polyclonal anti-H protein; N, monoclonal anti-N protein; M, polyclonal anti-M antibodies. P, H, N, M, and 32K refer to measles virus proteins obtained with mRNA from lytically infected cells. A, mRNA from lytically infected cells; B, brain tissue used as a control; C, patient 1; D, patient 2; E, patient 3; F, patient 4.

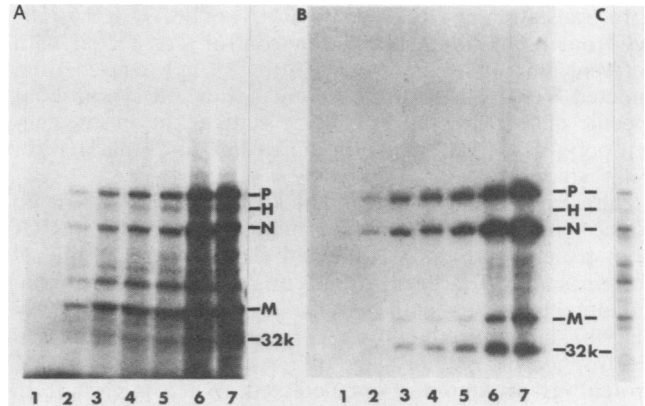


FIG. 3. Analysis of measles virus proteins synthesized in vitro by using different concentrations of mRNA. mRNA derived from lytically infected Vero cells (10, 25, 50, 100, 250, 500, and 1,000 ng, lanes 1 to 7, respectively) in 1 μ l of H₂O was incubated with 10 μ l of rabbit reticulocyte lysate for 30 min. Aliquots were either analyzed directly in a 10% polyacrylamide gel (A) or first immunoprecipitated with rabbit anti-measles hyperimmune serum (B). (C) The proteins made in vitro with a mixture of 25 ng of mRNA from infected cells and 500 ng of brain mRNA used as a control.

patient to patient but were generally low when compared with the amounts in the lytically infected cells. Particularly striking were small amounts of H and F mRNAs in patients 2 and 4; these mRNAs were only detectable after long exposures of the autoradiographs (Fig. 1D and F). In the Northern blot of oligo(dT)-selected RNA from patient 2, genome-size RNA can also be readily seen (Fig. 1D). In contrast to RNAs from other patients, this species was not removed even after three cycles of oligo(dT) chromatography. When different parts of each brain were examined, we observed variations only in the quantities of measles virus-specific RNA but no variations in the relative amounts and sizes of particular RNAs (data not shown).

Analysis of mRNAs in in vitro translation reactions. The measles virus proteins which could be detected by in vitro translation reactions by using viral mRNAs isolated from infected tissue culture cells are shown in Fig. 2A. By immunoprecipitation P, H, N, M, and a 32,000-molecular-weight protein (32K protein) could be identified. F protein could never be detected despite the application of a variety of monoclonal antibodies and a monospecific antiserum against this specific protein. The 32K protein produced during in vitro translation is possibly related to N protein since it was precipitated by a monoclonal antibody against N protein. However, this 32K protein was only seen in in vitro translation and not in infected tissue culture cells.

To establish which concentration of mRNA during in vitro translations is necessary for detectable synthesis of measles virus proteins, increasing amounts of mRNAs extracted from lytically infected cells were added to the rabbit reticulocyte lysates. The synthesis of measles virus proteins could be detected at a concentration as low as 25 ng/11 μ l of the assay volume (Fig. 3). At concentrations higher than 500 ng/11 μ l of the assay volume, the amount of polypeptides produced did not increase. Therefore, we used this concentration of brain RNAs to maximize the sensitivity of the in vitro translation experiments. Each in vitro translation of brain mRNAs was compared with the translation products of mRNA extracted from infected tissue culture cells. To investigate a possible inhibitory effect of brain mRNA on in

in vitro translation of virus-specific RNA, poly(A)⁺ RNA (500 ng) from brain tissue used as a control was mixed with different amounts of mRNAs (10 to 500 ng) derived from infected Vero cells. No interference with the synthesis of specific measles proteins could be detected at an infected-cell poly(A)⁺ RNA concentration as low as 25 ng/11 μ l of assay volume (Fig. 3C).

Different defects in the abilities of the brain RNAs to direct synthesis of four virus proteins were found. mRNA from patient 1 (Fig. 2C) directed the synthesis of the N protein and two more measles virus-specific proteins. Immunoprecipitations with specific antisera showed clearly that the larger product was P protein with an increased electrophoretic mobility and the smaller product was M protein; H protein was not detected. When mRNA from patient 2 (Fig. 2D) was translated in vitro, N protein was synthesized and also P protein, which migrated more slowly than the reference in this case. H and M proteins were not detected. mRNA from patient 3 (Fig. 2E) directed synthesis of N and P proteins, but H and M proteins were not found. In patient 4 (Fig. 2F) only N protein was detected in in vitro translations, although P and M mRNAs were present at concentrations similar to that of N mRNA as judged by Northern blot analysis. The same results were obtained by using mRNA from different parts of the brain of patient 4 (data not shown).

To test whether the measles virus genes in the SSPE patients were mutated or contained deletions which would have led to proteins no longer recognizable by the antisera shown in Fig. 2, we carried out immunoprecipitation of the in vitro translation products with the sera and CSFs of patients 1, 2, and 4. However, no additional bands were visible (Fig. 4).

DISCUSSION

We have recently described the expression of a defective measles virus M gene in the brain of an SSPE patient (4). In the present study we extended our analysis to other measles virus mRNA species and their translation in this particular SSPE case and have included three additional SSPE cases. All four SSPE-infected brains revealed the characteristic neuropathological changes, and the brain areas selected for RNA analysis contained measles virus antigens. By immunocytochemical staining with monoclonal antibodies to five structural proteins of measles virus (H, P, F, N, and M), P and N proteins were always detectable in brain sections, whereas the detection of the H, F, and M proteins varied considerably.

The results of the biochemical analyses shown here are complex. Generally, the mRNAs for N, P, and M proteins were easily detectable. Those for H and F proteins were always present but in lower amounts compared with lytically infected tissue culture cells. Although the absolute amounts varied, the relative amounts of the different virus mRNAs within different parts of the same brain were stable. The relative concentrations of H and F mRNAs were always rather low in comparison with the relative concentration of N, P, or M mRNA. This could have been due to either diminished transcription rates of F and H mRNAs or increased degradation of F and H mRNAs. All measles virus mRNAs could be found in three of the four SSPE-infected brains. In patient 3, no mRNAs for P and M proteins were detected. In this case, hybridization with cDNA clones to P and M message revealed a high amount of P-M bicistronic transcripts.

To see whether the mRNAs detected were able to direct synthesis of measles virus proteins, they were tested in an in vitro translation system which allowed the analysis of H, P, N, and M polypeptides. F protein could not be studied, since monoclonal and monospecific polyclonal anti-F antibodies in this study did not immunoprecipitate this protein from the in vitro products.

mRNAs isolated from all patients directed the synthesis of N protein. mRNA from patients 1 and 2 directed the synthesis of P proteins of different electrophoretic mobilities than those seen in lytic infection and in patient 3. Variation in the molecular weight of this protein has also been observed in persistent infections in cultured cells and seems to occur quite often when different measles virus strains are compared (23). In contrast, P protein could not be seen in the in vitro products of patient 4, although the presence of P protein in the brain was demonstrated by immunofluorescence. It is possible that the population of mRNAs present in patient 4 is heterogeneous and the majority of P mRNA is not translatable because it contains mutations. The small amount of functionally intact message may not be sufficient to direct the synthesis of detectable amounts of P protein in

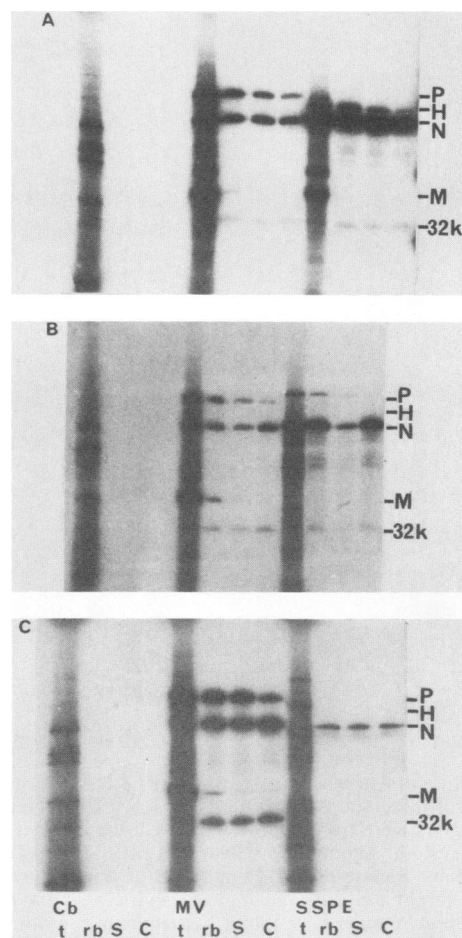


FIG. 4. Homologous immunoprecipitation of in vitro translation products. Polyadenylated RNAs were translated in vitro and either analyzed directly on a 13% sodium dodecyl sulfate-polyacrylamide gel or first immunoprecipitated with rabbit antimeasles hyperimmune serum (rb), serum (S), or CSF (C) of the corresponding SSPE patient. Cb, control brain tissue; MV, virus-infected Vero cells; SSPE, patients. A, patient 1; B, patient 2; C, patient 4.

TABLE 2. Summary of biochemical and immunohistological data^a

| SSPE patient | Measles virus mRNAs in brain tissue | | | | | In vitro translation products of brain RNA | | | | Measles virus structural proteins in brain cells | | | | |
|--------------|-------------------------------------|----|----|-----|-----|--|---|---|---|--|---|---|---|---|
| | N | P | M | F | H | N | P | M | H | N | P | M | F | H |
| 1 | + | + | + | (+) | (+) | + | s | + | - | + | + | + | - | - |
| 2 | + | + | + | (+) | (+) | + | l | - | - | + | + | - | + | - |
| 3 | + | bi | bi | (+) | (+) | + | + | - | - | + | + | - | + | + |
| 4 | + | + | + | (+) | (+) | + | - | - | - | + | + | - | + | + |

^a (+), Detectable but in reduced amounts; bi, bicistronic transcript; s, protein apparently smaller than normal; l protein apparently larger than normal.

the in vitro system. In vivo, this protein may accumulate in infected cells to levels detectable by immunofluorescence. The synthesis of P protein at low levels in patient 3 is probably directed by the P-M bicistronic transcripts.

Of particular interest is the result obtained for M protein in patient 1. In contrast to that in the other patients, M protein was detectable in patient 1 by in vitro translation of the mRNA as well as by immunofluorescence in infected brain cells. Obviously, there are distinct differences among the SSPE cases in the function of this mRNA. Transcription apparently occurred in most cases, but accumulating mutations may have prevented translation, as we have recently shown in patient 3 (R. Cattaneo, A. Schmid, G. Rebmann, K. Baczko, V. ter Meulen, W. J. Bellini, S. Rozenblatt, and M. A. Billeter, submitted for publication). In this case, sequence analyses of the M gene derived directly from a diseased brain reveals high rates of base transitions and transversions; a protein cannot be synthesized because one base substitution creates a translation stop at codon 12 of the reading frame. It is conceivable that similar changes may have developed in patients 2 and 4. However, mutations that interfere with M protein synthesis did not occur in every case, as is demonstrated by patient 1 and as has been shown by others by use of immunofluorescent staining techniques with monoclonal antibodies on SSPE-infected brain sections (21). However, it is unknown whether M protein is functionally competent in these cases.

Attempts to detect H protein in the in vitro translation products with mRNA isolated from an SSPE-infected brain were unsuccessful. This could have resulted from the relatively low concentration of this mRNA, as seen in the Northern blots, or it may be due to a functional defect of the mRNA. Defects in the synthesis and maturation of the H protein may be alternative mechanisms for the persistence of measles virus (16). Kimoto and Baba (17) describe a virus isolate from an SSPE patient, which remained cell associated and expressed only low amounts of H protein. Breschkin et al. (7) have isolated a cell-associated noninfectious virus from an SSPE patient which showed no hemadsorption because of a block of its insertion into the cell membrane. Young et al. (32), who investigated persistency with measles virus, have found in one cell line that the defect of H protein transport to the cell surface is due to changes in the sugar residues of the H protein. This alteration is sufficient to generate a nonproductive state of infection.

The results of the biochemical and immunohistological investigations are summarized in Table 2. The data presented demonstrate that measles virus persistence in SSPE-infected brain cells is characterized by restriction of several virus genes. The mRNAs for M, H, and possibly F proteins seem to be those most affected. The transcription of M mRNA obviously occurred in three patients, but the message was either not translated owing to the accumulation of mutations (Cattaneo et al., submitted) or if translated, the

protein synthesized was probably not functional, since the ultrastructural changes in the infected brain areas of patient 1 were not different from those seen in the other two cases (data not shown). These findings in SSPE-infected brain tissue are very similar to those observed in measles or persistent SSPE virus infections in tissue cultures. In some cell lines, M protein is either not translated (10) or is synthesized but subsequently degraded rapidly (28, 32), which results in a reduced intracellular pool of this protein. This phenomenon is not unique to measles virus persistence but may be common in persistent paramyxovirus infection, since it has also been observed in BHK cells persistently infected with Sendai virus (24). However, a nonproductive infection of measles virus does not depend alone on the M protein. Restriction in expression of the measles H gene in SSPE-infected brain tissue, as observed in some cases we studied, could certainly contribute to persistence; this probably would apply to the F protein as well.

The mechanisms which lead to these molecular changes are still unknown. It is conceivable that host factors play a role, since in some cases infectious SSPE virus can be recovered after cocultivation of brain cells with cell lines capable of supporting measles virus replication (31). On the other hand, RNA viruses have the tendency to accumulate mutations in persistent infections, and the mutated genomes survive (15), possibly because not all virus gene products are required and no selection pressure is operative for dispensable genes. In SSPE there is apparently no need for the production of infectious virus particles since the nucleocapsid alone is infectious (26). Therefore, mutations of measles virus genes, except for the N, P, and L genes, are not strictly deleterious for the survival of the persisting virus but may, on the contrary, enable the virus to escape the immune response. Further sequence analysis of the crucial measles virus genes in SSPE will show whether mutations are indeed accepted preferentially in M, F, and H genes and will provide some indications as to whether random mutation alone or other factors play a role in measles virus persistence in this disease.

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