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Measles virus gene expression in subacute sclerosing panencephalitis

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Summary

RNA was extracted from the diseased brain of a case of human subacute sclerosing panencephalitis (SSPE) and analysed for the expression of measles-specific RNA. Measles virus-specific mRNAs were present, but the amount of matrix (M) protein mRNA was greatly reduced in comparison to lytically infected cells and phospho- (P) protein mRNA was hardly detectable whereas the level of the corresponding intermediate-sized (is-) RNA was greatly increased. RNA obtained from the human brain was also translated in vitro and measles virus nucleocapsid and P protein was produced. However, in marked contrast to control reactions M protein was not detected in the products formed by translation in vitro. These results indicate an impaired measles virus M protein mRNA synthesis in infected brain tissue.

measles, subacute sclerosing panencephalitis, transcription, translation, matrix protein

Introduction

Subacute sclerosing panencephalitis (SSPE) is a slowly progressing, fatal disease of the human central nervous system associated with a persistent measles virus infection (ter Meulen et al., 1983). A characteristic finding in this disease is the presence of measles virus nucleocapsids in the cytoplasm and nucleus of infected

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brain cells in the absence of budding measles virus particles. Infectious virus does not appear in the brain of SSPE patients and isolation of SSPE virus can only occasionally be achieved by co-cultivating infected brain cells with susceptible primate cells. These observations point to a defect of measles virus replication in brain tissue, and recent studies have suggested that this defect may be related to lesions in the production of virus matrix (M) protein (reviewed by ter Meulen and Carter, 1982). Several immunological investigations have demonstrated that the majority of SSPE patients do not mount an immune response to measles virus M protein (Hall and Choppin, 1979; Hall et al., 1979; Stephenson and ter Meulen, 1979; Wechsler et al., 1979), which may result from a lack of M protein synthesis in SSPE brain (Hall and Choppin, 1981). This interpretation is further supported by studies of non-productive cell lines which, although persistently infected with SSPE viruses, do not express measles virus M protein (Lin and Thormar, 1980; Machamer et al., 1981; Carter et al., 1983a). In one of these cell lines the defect lay at the level of mRNA translation (Carter et al., 1983a).

In order to understand the pathogenic mechanisms involved in SSPE and the underlying virus–cell interaction in brain cells it is important to analyse measles virus replication in the brain tissue of SSPE patients. This is now possible in part since DNA copies of the mRNAs encoding the measles virus M, phospho- (P) and nucleocapsid (N) proteins have been recently cloned in plasmid vectors (Gorecki and Rozenblatt, 1980; Rozenblatt et al., 1982; Bellini et al., 1984). In addition, one clone has been obtained from the 3′ terminal region of the virus genome that overlaps with the cloned cDNA sequence corresponding to a part of the nucleocapsid protein mRNA (Billeter et al., 1984). We have therefore examined the nature and function of virus-specific RNAs in the brain of one SSPE patient and have found that the pattern of virus RNA transcription was strikingly altered. The M protein mRNA for measles virus was detectable but greatly reduced in SSPE brain and no M protein product could be detected by in vitro translation. These findings support the interpretation that in this SSPE patient measles virus replication in the brain was altered leading to an impairment of M protein synthesis.

Materials and Methods

SSPE patient

Patient K. was a fourteen-year-old boy suffering from SSPE for 18 months. Shortly after death the brain was removed and sectioned: some was processed for virological and pathological studies and the remainder was stored at $-70°C$. Histological examinations revealed the neuropathological changes characteristic of SSPE. Dense inclusion bodies consisting of measles virus nucleocapsid were present in the cells of the CNS. All attempts to isolate infectious measles virus from the brain tissue by the co-cultivation technique failed. For this study brain areas were selected which revealed Cowdry type A inclusion bodies.

Preparation of RNA samples

Vero cells were infected at a multiplicity of 0.01 with measles virus Edmonston

and harvested when cpe was complete. Cytoplasmic extracts were then prepared (Baczko et al., 1983) and total RNA extracted by the method of Chirgwin et al. (1979).

From 2 g of human brain, 150 μg of RNA was extracted also, by the method of Chirgwin et al., 1979. RNA derived from both sources was melted and subjected to poly-U sepharose chromatography. Poly(A)$^+$ fractions were precipitated twice with ethanol and adjusted to 1 mg/ml in water. Samples were then analysed by electrophoresis and blotting techniques, or tested for translation activity in vitro. Electrophoresis of RNA was performed using vertical 1% agarose gels containing 0.05 M boric acid, 0.005 M sodium borate, 0.01 M sodium sulphate, 0.001 M EDTA, 6 mM methylmercury hydroxide (Bailey and Davidson, 1976). RNA was denatured with 20 mM methylmercury hydroxide (5 min, 50°C) before application to the gel. Samples were electrophoresed for 3 h at 30 mA, and then transferred to nitrocellulose filters (Thomas, 1980). The filter was marked to enable re-alignment, and cut into strips, each of which was hybridised to a different ^{32}P-labelled (nick translated, 2–3 × 10^8 cpm/μg) cDNA prepared from measles virus mRNA or genome RNA.

Protein labelling

Translation reactions were performed using a rabbit reticulocyte lysate containing [^{35}S]methionine. Lysates were prepared by the method of Pelham and Jackson (1976), and kindly provided by Dr. S. Siddell, Würzburg. Proteins synthesized in infected Vero cells were labeled with [^{35}S]methionine for 2–4 h. Cells were then washed once with ice-cold PBS and about 5 × 10^6 cells were scraped into 5 ml of ice-cold RIPA-detergent buffer (150 mM NaCl, 10 mM Tris pH 7.4, 1% DOC, 1% Triton X-100, 0.1% SDS, 1 mM PMSF, 5% aprotinin), vortexed for 20 s and centrifuged for 20 min in a SW50 rotor at 0°C with 25 × 10^3 rpm. The supernatant cell lysate was stored at −20°C. Products formed by translation in vitro were diluted 10-fold in RIPA buffer containing 1 mM PMSF and 5% aprotinin and used directly. Immunoprecipitations were performed at 0°C using serum from rabbits immunized with measles virus Edmonston, or SSPE virus LEC. After 2 h of immunoprecipitation 20 μl of protein A–Sepharose CL-4B (Pharmacia Fine Chemicals) 50% (v/v) in RIPA was added per 100 μl reaction and incubated with shaking at 0°C. After 1 h the reaction mix was washed 3 times with RIPA-detergent mix. To the washed pellet 25 μl of 2 × sample buffer (Laemmli, 1970) was added, incubated at 100°C for 2 min, spun for 2 min in an Eppendorf centrifuge, and the supernatant was electrophoresed in a 10% polyacrylamide gel containing SDS, using the discontinuous buffer system of Laemmli (1970).

Results

Identification of measles antibodies in serum and CSF specimens

During the entire disease period the patient was in a state of hyperimmunisation toward measles virus and in the biological assays applied (haemagglutination inhibition, neutralization, complement fixation) high antibody titers to measles virus were

measured. Measles antibodies were present in both serum and CSF and their specificity was examined by immune precipitation (Fig. 1). This revealed a characteristic low response toward matrix protein in the serum, and antibodies to this protein were completely lacking in the CSF. CSF specimens always showed a marked IgG increase, with virus specific oligoclonal bands, whereas the total protein concentrations were normal.

Detection of measles-specific RNA in brain tissue

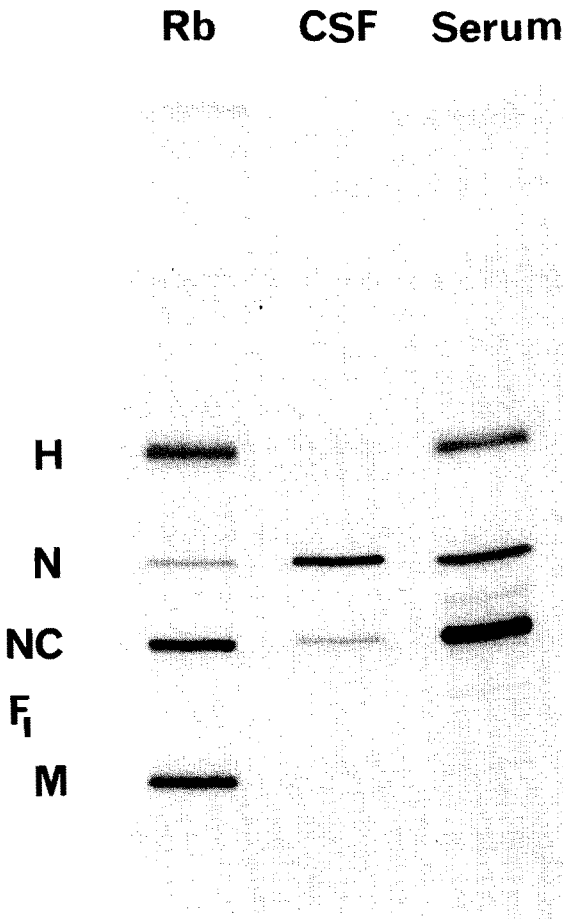Extracted brain RNA was analysed after poly(U)–Sepharose chromatography for

Fig. 1. SSPE patient immune response. Measles virus (Edmonston) proteins were immunoprecipitated from infected Vero cell lysates using: Rb, rabbit anti-measles Edmonston hyperimmune serum; CSF and serum sample from SSPE patient K. Radiolabelling of protein and immunoprecipitation were performed using [^{35}S]methionine as described in Materials and Methods. Proteins were separated on 10% polyacrylamide–SDS gels. Measles virus proteins observed: the haemagglutinin (H), the nucleocapsid protein (N) and its major degradation product (NC), the major cleavage product of the fusion protein, (F$_1$), and the matrix protein (M).

the presence of virus-specific mRNAs by the blotting technique. Strips of the filter were hybridised to individual, ^{32}P-labelled measles virus-specific cDNA clones (Fig. 2). Three size classes of measles virus-specific RNA are recognized inside infected cells (Carter et al., 1983a). The largest is thought to consist of genome, and the smallest, most abundant, of mRNA. The intermediate-sized (is-) RNA is probably a readthrough transcript of two adjacent genes (Baczko et al., 1984) as observed in other paramyxovirus infected cells (Collins and Wertz, 1983). These is-RNAs have been used to deduce the probable arrangement of genes on the 50 S RNA genome (Baczko et al., 1984). Genomic RNA cannot be seen in Fig. 2 because it is removed by poly(U)Sepharose chromatography. mRNAs for three different virus-specific proteins P, N and M, and several is-RNAs were detected in the poly(A)$^+$-RNA extracted from infected cells (Fig. 2a, b). Intermediate-sized RNA was only visible on prolonged exposure (Fig. 2b). In the polyadenylated RNA fraction isolated from
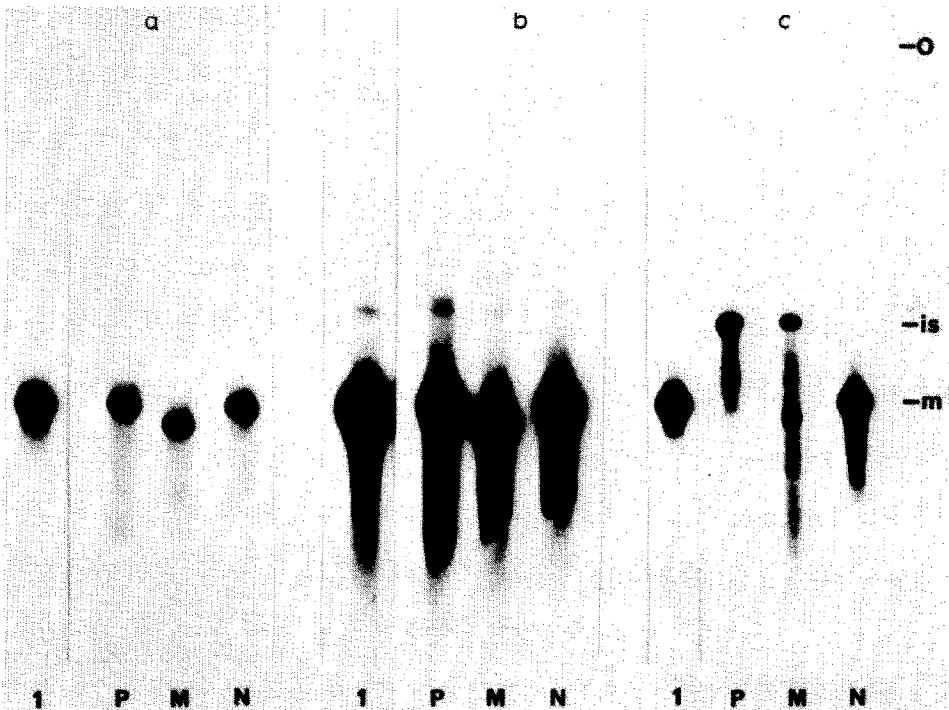

Fig. 2. Analysis of measles virus specific RNAs by filter hybridisation. RNAs were extracted from with Edmonston virus-infected cells or SSPE brain of patient K. From 50 μg of total RNA, poly(A)$^+$-RNA was selected, subjected to electrophoresis and blotted onto nitrocellulose. 3 mm strips of the resulting filter were hybridised to: 1, 3' end of the genome and part of the nucleocapsid protein gene (Billeter et al., 1984); P, phosphoprotein gene; M, matrix protein gene; N, nucleocapsid protein gene (Gorecki and Rozenblatt, 1980; Rozenblatt et al., 1982; Bellini et al., 1984). O, origin; is, intermediate-sized RNA; m, mRNA. (a) Polyadenylated RNAs from Vero cells lytically infected with measles virus Edmonston; exposure time was 20 h. (b) Same as a, with an exposure time of 4 days. (c) Polyadenylated RNA from SSPE brain; exposure time was 14 days.

SSPE brain (Fig. 2c) the three virus-specific mRNAs could also be detected. However, whereas mRNAs for P and M proteins are usually abundant in infected tissue cultures and greatly exceed in amount the various is-RNA species (Fig. 2b), this balance was disturbed in RNA from SSPE brain. The mRNA for P protein was hardly detectable and mRNA for the M protein was only found in low amounts. In contrast, the is-RNA detected with both P- and M-specific probes, and thought to represent read-through transcripts of these two adjacent genes, was increased and detected in amounts which exceeded those of the mRNAs. This phenomenon could be accounted for by a failure to terminate the mRNA correctly at the boundary of the P and M genes. When RNA from lytically infected cells was analysed by this technique, M protein-specific clones detect two is-RNA bands. These correspond to the readthrough transcription products of the P and M genes and also M and its 5′ neighbour on the genome. In SSPE brain, only the P-M is-RNA is observed. No virus-specific RNA could be detected in control brain (data not shown).

In vitro translation reactions of isolated brain RNA
    The function of the mRNAs was investigated by in vitro translation, using a reticulocyte lysate system, and compared with the translation products of mRNA extracted from lytically infected Vero cells. Fig. 3 illustrates the products of this
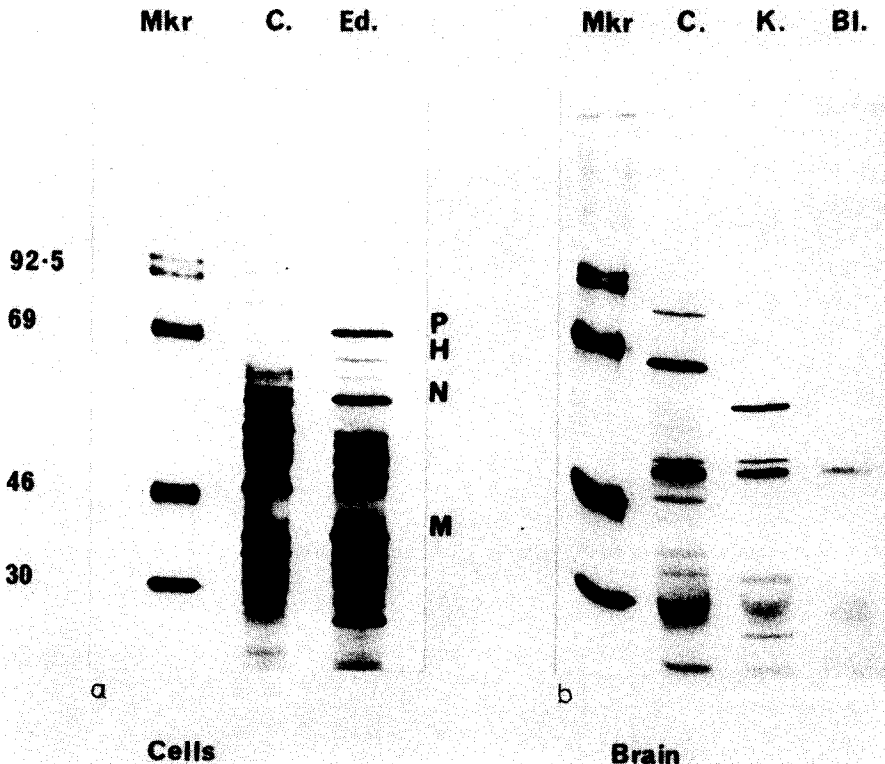
reaction. P, H and N were clearly observed, but M couuld only be identified with certainty after immunoprecipitation (Fig. 3c). All virus-specific proteins except L and F could thus be identified in the translation products of infected cell mRNA. The unmodified translation product of the H-specific mRNA has a molecular weight of 65k (Bellini et al., 1983) and is very poorly recognised by the antiserum. In addition, large amounts of nucleocapsid (57k) and matrix protein (38k) were formed. The prominent protein product of molecular weight 32k, related to the N protein (Rima et al., 1981), which we term X is also detected in infected cell lysates and in the in vitro translation products of mRNA isolated from such cells (Carter et al., 1983a).
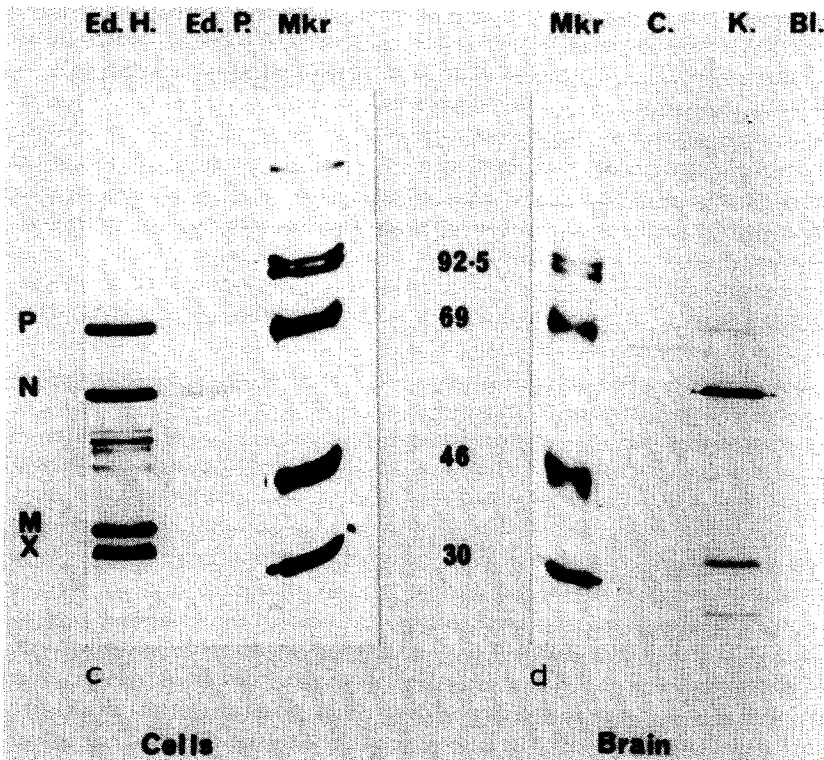

Fig. 3. In vitro translation of mRNAs. RNAs were extracted, poly(A)-selected and 1 μg aliquots were translated in a rabbit reticulocyte lysate system (provided by Dr. S. Siddell). Aliquots of the products were diluted in RIPA buffer containing protease inhibitors and immunoprecipitated using rabbit anti-measles virus hyperimmune (Ed.H) or preimmune (Ed.P.) serum, preadsorbed with uninfected Vero cell antigens. Samples were analyzed on a 10% SDS–polyacrylamide gel and fluorographed. MKR, molecular weight markers. (a) Total in vitro translation products using mRNA from uninfected Vero cells (C) and infected Vero cells (Ed.). (b) Total in vitro translation products using mRNA from human control brain (C), SSPE brain (K) and blank without mRNA addition (Bl). The control brain was obtained from a 20-year-old person who died in a traffic accident. (c) Immunoprecipitation of in vitro translation products from infected Vero cells using hyperimmune and preimmune serum. (d) Immunoprecipitation of in vitro translation products of human SSPE brain RNA and negative control, with hyperimmune serum.

RNA isolated from the SSPE patient brain gave rise to large amounts of nucleocapsid and X protein products (Fig. 3a, b). A small amount of P protein was also produced (Fig. 3b) which has been identified in another experiment with a monospecific antiserum prepared against a synthetic peptide of measles virus P protein (data not shown; Bellini et al., 1984). We were unable to detect the synthesis of the H and F proteins. These regions of the gel were obscured by host proteins and the unmodified protein cores of both of these glycoproteins were recognised poorly by all sera available to us. The M region of the gel was not obstructed, but nevertheless M protein could not be detected amongst the translation products of the RNA extracted from SSPE brain either before (Fig. 3b), or after (Fig. 3d) immunoprecipitation. The X polypeptide was immunoprecipitated, and clearly differentiated from M, by reference to the molecular weight markers. M-specific mRNA was present in relatively low amounts, and it was therefore possible that this could explain our failure to observe production of this protein. This possibility was tested by translating RNA samples in which the amount of M protein RNA had been equalised to that in controls. This was performed by serial dilution of control RNA until levels of the M-specific message were equal as judged by Nothern blotting. However, these experiments were inconclusive since it was difficult to identify unequivocally M protein in the translation products of the diluted mRNA.

Discussion

In this report we have described the presence of three measles virus-specific mRNAs in the brain of an SSPE patient and have examined the activity of these mRNAs in in vitro translation reactions. The N and P proteins were readily produced by these techniques which was surprising for P protein in view of the low levels of the corresponding mRNA in infected brain. However, it is possible that the 5' reading frame of the is-RNA is able to function in translation reactions, as with other structurally polycistronic (coronavirus) mRNAs (Siddell et al., 1983). In contrast, the mRNA of the M protein, which was also only detectable in low amounts in SSPE brain failed to give rise to a specific product although the matrix protein was formed in large amounts by control reactions. This failure could result from an alteration of this specific mRNA which would prevent correct function in translation reactions, as studies of non-productive cell lines persistently infected with SSPE virus indicate (Carter et al., 1983a). Alternatively, quantitative reduction of this mRNA could also be sufficient to account for this observation as the dilution experiments may suggest. At the present time we cannot decide which mechanism had led in this SSPE case to the impairment of the M protein synthesis.

Since we were unable to demonstrate the formation of H or F proteins in an in vitro translation assay, or to investigate the synthesis of their mRNAs, it is possible that a defect could exist in the production of either or both of these proteins. However, H and F are difficult to detect even in translation reactions utilising mRNA from productively infected cells. This could be due to poor utilisation of these mRNAs by the translation system or to a low methionine content in the

proteins. Patient K. synthesised high levels of antibodies detectable in serum specimens which were directed to the H protein and could be demonstrated in haemagglutination inhibition and immune precipitation procedures. This observation suggests that haemagglutinin was probably produced in the patient and was able to stimulate the immune system. Since specific cDNA clones representing sequences of the mRNAs for measles virus H protein have not been unequivocally identified we were unable to study this aspect further.

The failure to produce the correct M protein product in detectable amounts in SSPE brain is thought to account for the absence of mature infectious virus in infected brain. M protein is thought to play a vital role in particle assembly, both in packaging of the nucleocapsids and in budding (Simons and Garoff, 1980). Lesions in this process would support virus persistence and reduce the spread of virus within brain tissue thus resulting in a slowly progressing disease course. However, the occasional isolation of infectious SSPE virus by co-cultivation techniques (Katz and Koprowski, 1973) suggests that this defect can either be overcome or is not universal. Other workers (Herman et al., 1975) have identified readthrough transcription of adjacent genes on the VSV genome. In this case the efficiency of the process was not totally governed by the template, but was also influenced by the polymerase. This observation could provide a clue to the mechanisms underlying the reversal of the block in M protein synthesis which presumably occurs in the occasional rescue of infectious virus by co-cultivation techniques (reviewed by ter Meulen et al., 1983), or physiological trauma (Carter et al., 1984). Both of these could affect the polymerase protein and act to restore correct transcription of the M gene. It is known that virus genes rapidly acquire mutations during persistent infection (Holland et al., 1979; Carter et al., 1983b). Some of these might damage the ability of the mRNA to function correctly even if its expression was restored, thus SSPE virus rescue would not be a universal phenomenon and the situation observed in some SSPE cell lines could thus be generated (Lin and Thormar, 1980; Machamer et al., 1981; Carter et al., 1983a).

Studies on additional SSPE cases including so-called acute SSPE (Gilden et al., 1975), as well as cloning and sequencing of measles virus M protein mRNA and is-RNA from SSPE brain, will provide valuable information on the nature of the impaired synthesis of matrix protein in relation to the disease process.

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