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SYNTHESIS OF MATRIX PROTEIN IN A SUBACUTE SCLEROSING PANENCEPHALITIS CELL LINE [1]

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INTRODUCTION

Subacute sclerosing panencephalitis (SSPE) is a slowly progressing, fatal disease of the human central nervous system (CNS), associated with measles virus persistence. The disease is characterised by an elevation of anti-measles antibody titres in both serum and CSF (1). Virus nucleocapsids are present as inclusion bodies in the cells of the CNS (2) but budding virus has not been observed and infectious particles have never been detected. The patient shows a low immune response toward matrix (M) protein, and direct examination of infected brain has failed to detect this molecule (3,4). All this evidence points to a defect in virus maturation which is associated with a lesion in the production of M protein. These features of SSPE have been reviewed (5,6). The defect in virus maturation may be to some extent host-controlled, since co-cultivation, or fusion of cells from infected tissue, with cell lines susceptible to measles infection, has sometimes resulted in the rescue of an infectious budding virus (7). More often virus rescue is only partially successful and a cell-associated cytopathic agent is obtained. These cell lines are known as SSPE cell lines, and many reports have confirmed that they also possess a lesion in the synthesis of M protein (8, 9). We have recently described two such SSPE cell lines established by co-cultivation of SSPE patient brain with Vero cells, in which matrix protein was not produced (10). In one (N-1), mRNA specific for M protein was present but unable to give rise to the normal protein by translation (11). In a second cell line (MF), we were unable to

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detect mRNA sized molecules with matrix protein-related sequences in the polyadenylated RNA fraction. We therefore concluded that failure to produce M protein in the MF cells was accomplished by a defect in mRNA formation or stability (10).

The MF cell line is unusual in that morphological features normally associated with virus maturation have been reported (12). Both the formation of an "inner leaflet" associated with M protein combination with the plasma membrane (13), and budding particles have been observed. These were found to be non-infectious (12). Our previous study (10) utilized high-passage MF cells (above 120) and we were therefore interested in examining this apparent maturational phenomenom in relation to the expression of M protein in early passage cells.

RESULTS

MF cells were revived from liquid nitrogen storage at various passage levels. We examined A(+) RNA, prepared from these cells, by the technique of Southern blotting using nick-translated plasmids containing M protein mRNA sequences (14). A messenger RNA molecule carrying matrix protein sequences was found to be present in one of these cultures (passage 60: figure 1A, track 1) and it was of similar size to that observed in a productive Edmonston virus infection (track 2). We have described another SSPE cell line (N-1) in which M protein mRNA was readily detectable but not utilized correctly in translation reactions, thus it was possible that the mRNA detected in MF cells might not function in protein synthesis. We therefore used the A(+) RNA fraction analysed in Figure 1A, in an *in vitro* translation reaction. The virus-specific products of this reaction were identified by immune precipitation and separated on an SDS polyacrylamide gel (figure 1B). Polyadenylated RNA extracted from a productive measles virus infection was used as a positive control , and from uninfected Vero cells as a negative control. M protein was detected among the translation products of both MFand Edmonston virus-infected cell mRNAs. Thus we conclude that this mRNA was functional *in vitro*.

The lesion in M protein production within SSPE patient brain is known to be to some extent host-controlled since cell-fusion or co-cultivation experiments have resulted in the expression of this protein (7). A host- controlled defect in translation has been postulated in a laboratory-produced SSPE virus persistent infection, since matrix protein produced by *in vitro* translation could not be detected within the persistently infected cells (5). We therefore prepared 35-S methionine labelled extracts from these early passage cells and from uninfected Vero cells and examined them for the presence of

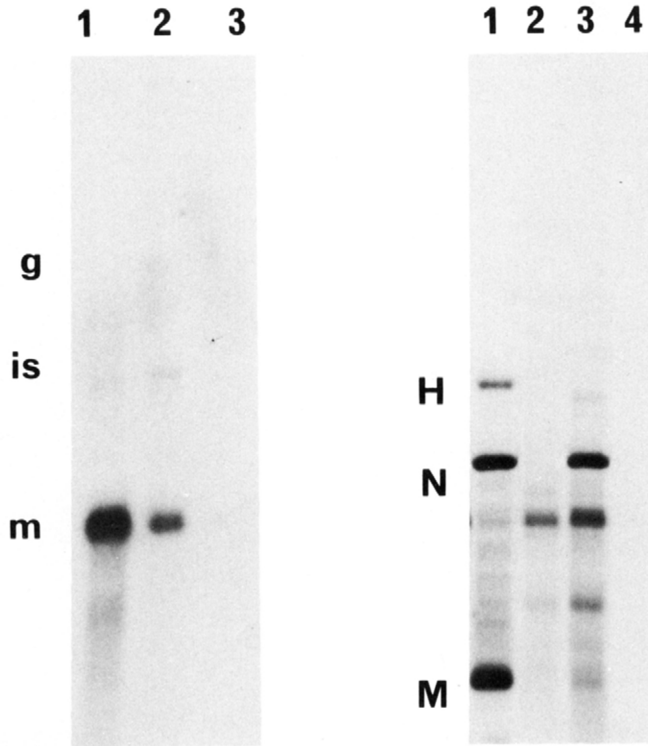

Figure 1.
Panel A. Polyadenylated RNA was extracted from:track 1, Edmonston virus-infected Vero cells; track 2, MF cells; track 3, uninfected Vero cells, and separated by electrophoresis on a Mops buffered 1.5 % agarose gel. RNA was transferred to a nitrocellulose filter and hybridised to 32-P labelled nick-translated plasmid containing sequences derived from the matrix protein mRNA. (g), genome; (is), intermediate-sized RNA; (m), mRNA.
Panel B. Immunoprecipitated protein products formed by translation of the mRNAs analysed in panel A. track 1, Edmonston virus-infected Vero cell mRNA; track 2, uninfected Vero cell mRNA; track 3, MF cell mRNA; track 4, No mRNA addition. Haemagglutinin (H), Nucleocapsid protein (N) and matrix protein (M) were identified by reference to molecular weight markers.

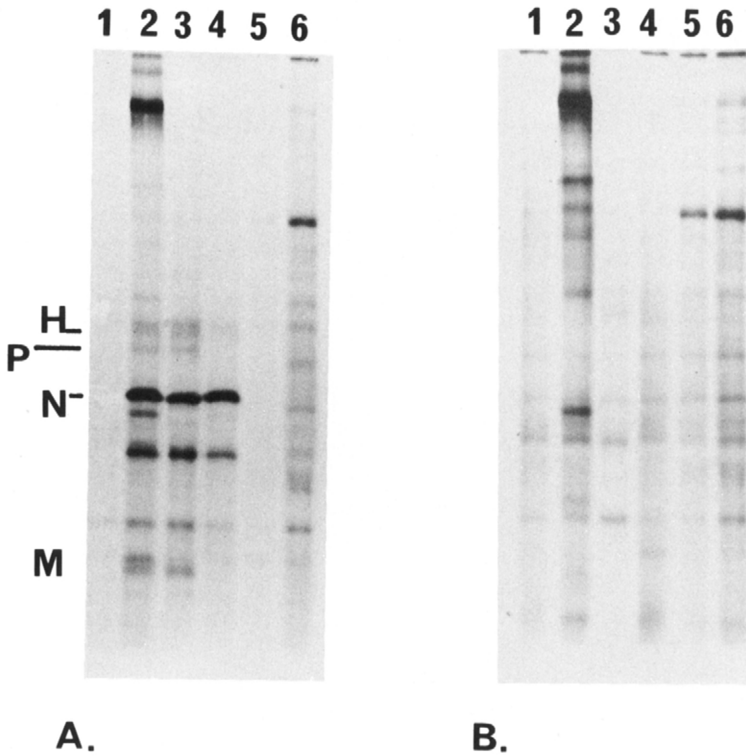

Figure 2.
Immunoprecipitation of proteins from cell lysates. MF cells (panel A) and uninfected Vero cells (panel B) were labelled with 35-S methionine and processed for immunoprecipitation. In each panel proteins were immunoprecipitated by: 1, preimmune rabbit serum; track 2, hyperimmune rabbit anti-measles virus serum; track 3, serum from patient MF; track 4, CSF from patient MF; track 5, control monoclonal antibody; track 6, monoclonal antibody directed against measles virus M protein.

matrix protein by immune precipitation (figure 2). Serum and CSF are still available from the patient from whom the MF cell line was originally derived, and these were used in conjunction with rabbit hyperimmune anti-measles virus serum, and a monoclonal antibody specific for measles virus matrix protein. Rabbit preimmune serum and a monoclonal antibody directed against corona virus antigen provided negative controls.

Matrix protein was produced by the MF cell line and was immunoprecipitated by both the hyperimmune rabbit and human sera. It was not however detected using the monoclonal antibody, or the human CSF. The failure of CSF to immunoprecipitate this peptide was expected since the MF patient suffered from clinically normal SSPE, and antibodies to this protein are lacking in the CSF. We therefore concluded that in this culture of SSPE MF the production of mature functional mRNA for matrix protein led to the successful expression of M protein. In other cultures this protein was not detectable presumably due to the lesion in the production of the corresponding mRNA previously reported (10).

DISCUSSION

The results presented here raise several interesting questions. Firstly the production of M protein during the early passage MF cells that can be inferred from the previous morphological description, is supported by the immunoprecipitation of a matrix protein sized polypeptide formed in infected cells and in <u>in vitro</u> translation reactions. However this protein failed to react with monoclonal antibody directed towards measles virus Edmonston matrix protein. The reasons for this are unclear but this situation could arise from strain differences between the virus which originally established SSPE in the patient, and the Edmonston virus used for monoclonal antibody production. Such differences have been detected between measles virus haemagglutinin proteins by a variety of biological techniques (16). It seems likely that more than one strain of measles virus is capable of giving rise to SSPE, and we have observed nucleoproteins synthesised <u>in vitro</u>, using RNA extracted from two SSPE patients brains, to be of different sizes, a finding we also interpret in terms of virus strain differences .Alternatively this failure to react with monoclonal antibody could have arisen from mutations accumulating during persistence (17), and which have already been demonstrated in the measles virus matrix protein (18).

Budding particles have also been demonstrated in the early passage MF cells (12) but these were found to be non-infectious. It is generally assumed that restoration of matrix protein synthesis is all that is required during attempts to

rescue virus from SSPE patient brain, but the observations reported in the early characterization of the MF cell line suggest this may not be the case. Another SSPE cell line is known in which a lack of infectious virus was associated with a defect in H protein expression on the cell surface (9). Defective expression of H protein activity was also observed in the early passage MF cells (12). We have also observed a marked variation in the levels of nucleocapsid protein and its mRNA at various passages. Presumably adequate synthesis of all virus proteins is required for successful release of infectious particles. The MF patient lacked antibodies in the CSF directed against M protein, therefore this protein was probably not produced within the brain. Perhaps this block was overcome when tissue was co-cultivated with Vero cells and the maturation process previously described (12) could then occur. However, cells are not available from the passages used in that report. We observed only one MF culture in which matrix protein was produced. Furthermore other samples of the same passage cells failed to produce M protein when revived. However the data reported here indicate that MF cells possess the capacity to produce a mRNA which is active in translation. The mechanism which normally prevents the expression of this capacity is unknown, but it is possible that physiological trauma involved in the attempts to rescue virus from infected tissue, or cell revival from liquid nitrogen storage, is important in the successful circumvention of this block.

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