Measles and subacute sclerosing panencephalitis virus proteins: Lack of antibodies to the M protein in patients with subacute sclerosing panencephalitis

(Paramyxoviruses/persistent infection/viral pathogenesis/membrane proteins/protein synthesis)

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Contributed by Purnell W. Choppin, February 12, 1979

ABSTRACT  Studies on the immunoprecipitation of the proteins of several different measles and subacute sclerosing panencephalitis (SSPE) virus strains from lysates of infected cells have revealed a relative lack of antibodies to the nonglycosylated viral membrane protein (M) in the sera of patients with SSPE, in contrast to a high level of antibodies to other viral proteins. No evidence was found for the existence of a large number of antigenically unique M proteins. In contrast to SSPE sera, other hyperimmune human or rabbit sera precipitated M protein as well as the other viral proteins. The results suggest that in patients with SSPE there is either diminished synthesis of M, or it is not recognized normally by the immune system, and that an abnormality in M protein is involved in the pathogenesis of SSPE. Although differences in electrophoretic mobility of the M proteins and several other viral proteins were observed among different SSPE and measles strains, there was no pattern characteristic of SSPE strains, nor could these strains be distinguished by peptide mapping.

Subacute sclerosing panencephalitis (SSPE) is a slowly progressing disease of the central nervous system in which measles virus has been implicated as the causative agent. SSPE patients have high levels of antibodies to measles virus in their serum and cerebrospinal fluid, and "measles-like" viruses have been isolated by cocultivation of brain cells with cell lines (1, 2). (Isolates from patients with SSPE will be designated "SSPE strains" and those from patients with measles, "measles strains.") Because SSPE is a rare complication of measles, it is thought that unusual features of the virus or host or both play a role in pathogenesis. No consistent evidence for the involvement of genetic factors or immunological deficiencies has been found. Comparisons have been made of the biological and biochemical properties of SSPE and measles strains (1, 3-6) to investigate the role of the virus in the pathogenesis of SSPE. Measles virions contain six major polypeptides with molecular weights of ≈ 37,000-200,000 (7-9). These have been designated (8): L; H, the hemagglutinin; P, a nucleocapsid-associated protein; NP, the major nucleocapsid structural protein; F, a surface protein with hemolytic and cell-fusing activities, consisting of two disulfide-bonded subunits (F1 and F2); and M, a nonglycosylated membrane protein. In addition, the virus contains small and varying amounts of host cell actin.

In 1974 Schuenderberg et al. (3) reported that the M protein of an SSPE strain migrated more slowly on polyacrylamide gels than that of a measles strain, and Wechsler and Fields (5) recently reported that the M proteins of five SSPE strains migrated more slowly than that of one measles strain (Edmonston). In addition, Hall et al. (6) found with monospecific sera that the M proteins of one SSPE strain and the Edmonston strain were antigenically distinguishable, and that the smallest messenger RNA in cells infected with an SSPE strain, thought to be the messenger for M, migrated more slowly than the corresponding RNA in cells infected with a measles strain (6). These results suggested that SSPE strains may be mutants of measles virus, with the mutation affecting the M protein.

This report describes comparative studies of the proteins of several different SSPE and measles strains. We have studied the synthesis of viral proteins in infected cells and compared their electrophoretic mobilities. We have compared the M proteins of different strains by peptide mapping and investigated the ability of antibodies in the sera of SSPE patients and other hyperimmune sera to precipitate the proteins of SSPE and measles strains. This work was presented in part at the 4th International Congress for Virology, The Hague, Aug. 30-Sept. 4, 1978.

MATERIALS AND METHODS

Cell Culture and Viruses. CV-1 cells (TC7 clone) were grown in reinforced Eagle's medium containing 10% fetal calf serum as described (8). Three measles (Edmonston, Braxator, and 16-77) and three SSPE strains (Lec, Mantoofth, and Berg) were used in comparisons of viral polypeptides. In one experiment, an additional SSPE strain (HK) kindly provided by F. E. Payne (Ann Arbor, MI) was used. Cells were inoculated with undiluted virus at multiplicities of 0.05-10 plaque-forming units per cell, depending on the strain, and maintained at 37°C in medium without serum.

Isotopic Labeling of Infected Cells. Medium was removed when 60-70% of the cells had been incorporated into syncytia. It was replaced with methionine-free medium, and after 1 hr at 37°C [35S]methionine (50 μCi/ml; 1 Ci = 3.7 × 1010 becquerels) was added. After 3 hr cells were lysed and subjected to polyacrylamide gel electrophoresis or immunoprecipitation as described (10).

Sera. Sera from 28 different SSPE patients were kindly provided as follows: serum 1 by J. Zabriskie (New York); 2-4 by V. ter Meulen (Wurzburg, West Germany); 5 and 6 by P. Lachmann (Cambridge, England); 7 and 8 by F. E. Payne (Ann Arbor, MI) (serum 8 is HK serum); 9 and 22-25 by M. Oldstone (La Jolla, CA); 10-21 by G. Agranostotit (London); and 26-28 by K. Johnson (San Francisco). Sera from five patients with atypical measles A1-A5 and two sera taken before and after vaccination were provided by M. Kaplan (East Meadow, NY). Sera N2 and N3 were obtained from healthy children, and N1 was from an adult 22 years after uncomplicated measles. Hyperimmune rabbit sera prepared against purified measles (Edmonston) and SSPE (Lec) strains were also used.

Polyacrylamide gel electrophoresis, tryptic peptide analysis, autoradiography, and fluorography were carried out as described (10).

Abbreviation: SSPE, subacute sclerosing panencephalitis.
RESULTS

Polypeptides Synthesized in Infected Cells. To compare the synthesis of viral proteins in cells infected with SSPE and measles strains and their electrophoretic mobilities, CV-1 cells were infected with three measles (Edmonston, 16-77, Braxator) and three SSPE (Berg, Lec, and Mantooth) strains, and 18–54 hr after infection proteins were extracted and subjected to polyacrylamide gel electrophoresis. Because of the slow growth of some strains, not all of the viral polypeptides could be identified over the cell background in every case; however, the polypeptides of most interest in this work (M, NP, and P) were always identified (Fig. 1). No significant differences in protein synthesis were found among the strains. Although the M proteins of two SSPE strains (Mantooth and Berg) migrated more slowly than that of the Edmonston strain, the Lec SSPE strain and the Braxator measles strain migrated identically, and the 16-77 measles strain had the most slowly migrating M protein of all. Thus, there was considerable variation among strains, but the M proteins of the SSPE viruses did not always migrate more slowly than those of measles strains. Furthermore, Fig. 1 shows that the NP and P proteins of the six strains also varied significantly in migration, but there was no consistent pattern characteristic of measles versus SSPE strains.

Peptide Maps of M Proteins. Two-dimensional peptide maps of the M proteins from cells infected with the above six strains showed remarkable similarity (Fig. 2). All six M proteins contained three characteristic peptides (A, B, C), but, with the Edmonston strain, the middle spot in this region (B) had a different mobility, and another difference was also observed (spot D). Thus, although the Edmonston strain could be differentiated from the others, the M proteins of SSPE and measles strains could not be distinguished by this method.

Immunoprecipitation of Viral Proteins. To investigate the presence of antibodies to specific viral proteins in patients with SSPE, lysates of cells infected with the Edmonston strain were immunoprecipitated with nine SSPE sera, a serum obtained 22 years after uncomplicated measles, convalescent serum from a patient with atypical measles following vaccination with killed vaccine, and rabbit sera prepared against measles (Edmonston) and SSPE (Lec) viruses (Fig. 3 left). Whereas the rabbit and human atypical measles sera precipitated all the viral proteins, the SSPE sera precipitated very little or no M, even though these sera precipitated large amounts of the other viral proteins. The M precipitation by the SSPE sera was similar to, or less than, that obtained with the serum obtained 22 years after measles. These observations were extended (Fig. 3 center) with nine other SSPE sera (sera 10–18), and again very low levels of M were precipitated in most cases. Sera 16 and 17 are exceptions in that a significant amount of M was precipitated, but still less than the amounts of the other viral proteins. Of 28 SSPE sera tested, only 4 have precipitated M protein to appreciable levels. It should be indicated that, if the autoradiograms are exposed for very long periods, most of the sera show minimal amounts of M precipitation, amounts similar to or less than that obtained with normal sera in which antibodies to all the proteins are present at low levels. Thus, there is not a total absence of antibodies to M, but rather a great relative deficit. Indeed, it would be expected that some antibodies to M would be present arising from the initial measles infection that led to SSPE. In Fig. 3 center an additional polypeptide that migrates slightly behind the M protein is also seen. In Sendai virus-infected cells a polypeptide (B) migrating similarly has been shown to be a phosphorylated form of M (11).

In these experiments the sera were used undiluted; however, experiments were also done in which the sera were serially diluted, and there was a decrease in the precipitation of proteins with dilution. The atypical measles serum A1 still precipitated all the proteins, including M, at a dilution of 1:10,000, and three SSPE sera tested in serial dilutions precipitated all the proteins except M at this dilution. In those instances in which undiluted SSPE sera precipitated a small amount of M, there was no precipitation at dilutions >1:20.

Comparison of SSPE Sera with Other Measles Hyperimmune Sera. Because of the high levels of antibodies to proteins other than M in SSPE sera, it was appropriate to compare further these sera with other hyperimmune measles sera. Fig. 3 right shows the proteins precipitated by convalescent serum from three patients with atypical measles (A1, A2, and A3) and the acute and convalescent sera from two other such patients (A4a, A4c, A5a, A5c). The antibody responses in all convalescent sera were very high and all the viral polypeptides, including M, were precipitated. A similar precipitation of all polypeptides was seen in the postvaccination sera (V1, V2) of two children as compared to prevaccination sera (B1, B2). These children had presumably had measles before vaccination. Three additional SSPE sera (sera 19–21) are also shown in Fig. 3 right, and as before all precipitated large amounts of all of the viral proteins except M. Thus, in contrast to SSPE, under conditions in which

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**Fig. 1.** Polyacrylamide gel electrophoresis of the polypeptides synthesized in CV-1 cells infected with measles and SSPE strains and labeled for 3 hr with [35S]methionine. Measles strains: 16-77, Braxator (Brx), and Edmonston (Edm). SSPE strains: Lec, Mantooth (Man), and Berg. U1, uninfected cells.

**Fig. 2.** Tryptic peptide maps of the M proteins of measles and SSPE strains. M protein bands were excised from dried gels such as shown in Fig. 1, digested with trypsin, and analyzed by two-dimensional separation on cellulose thin-layer plates; 20,000 cpm of each digest was used.

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a vigorous antibody response was mounted to measles virus, antibodies to M as well as to the other viral proteins were produced.

**Immunoprecipitation with Different Virus Strains.** The above studies were carried out with cells infected with the Edmonston strain. To exclude the possibility that all the SSPE sera were failing to precipitate M simply because each patient had been infected with a virus whose M protein was antigenically distinct from that of Edmonston, similar studies were done with five other viruses [three SSPE strains (Berg, Lec, and Mantooth) and two measles strains (16-77 and Braxator)]. In each case, results similar to those above were obtained; the SSPE sera efficiently precipitated all the other viral proteins, but little or no M, and the atypical measles and rabbit sera precipitated all the proteins, including M. Fig. 4 shows examples of these studies with the 16-77 measles and Berg SSPE strains. Thus, the SSPE sera failed to precipitate the M proteins of three different SSPE and three measles strains, indicating that the phenomenon is independent of strain. These results do not exclude minor antigenic differences among strains.

The unlikely possibility could still be raised that there are a great many different SSPE strains, each with an antigenically different M, even though their other proteins were similar, and that none of the 28 patients tested were infected with a strain detectably similar to those used above. This was excluded by testing serum from an SSPE patient (lane 8 in Fig. 3) against the virus isolated from that patient. This matched serum–virus pair was generously supplied by F. E. Payne. As shown in Fig. 5, this SSPE serum precipitated large amounts of the other viral proteins, but very little M, whereas the atypical measles serum precipitated all the viral proteins. Thus, the above findings cannot be explained by extensive antigenic differences among the M proteins of different SSPE strains, and the results strongly suggest that there are very low levels of antibody to M protein in the sera of SSPE patients.

**M Protein Not Precipitated by SSPE Sera Can Subsequently Be Precipitated by Other Antibodies.** Experiments were done to rule out trivial or technical explanations—e.g., that some component in SSPE sera destroyed the M protein, or modified or blocked its antibody binding sites, or that the M protein was selectively lost during the procedure when SSPE
The finding of little antibody to M in the sera of SSPE patients suggests that this protein plays an important role in pathogenesis. It was previously shown that SSPE patients have high levels of hemagglutination-inhibiting, hemolysis-inhibiting, and neutralizing antibodies (now known to be directed against the surface glycoproteins, H and F) and also antibodies to the nucleocapsid protein (NP), and it had been estimated that 10–20% of serum IgG is measles virus specific (19). In addition, the finding of oligoclonal antibodies to measles antigens in the cerebrospinal fluid suggested a high level of antibody production in the central nervous system (14). Our studies have shown that in addition to antibodies to H, F, and NP, antibodies to the P and L proteins are present. In view of the vigorous response to the other viral proteins, the relative lack of antibodies to M in SSPE takes on added significance, because in other conditions in which there is a hyperimmune response to measles virus proteins—i.e., atypical measles following vaccination with inactivated vaccine or vaccination of immune patients—there is a response to M equivalent to that to other proteins. The relative lack of precipitation of M by SSPE patients’ sera was so consistent that this would appear to offer a simple diagnostic test for SSPE.

To establish that the minimal precipitation of M by SSPE patients’ sera was due to a lack of antibody rather than the existence of a great many unique antigenic types of SSPE M proteins that did not crossreact, extensive studies were carried out with four different SSPE strains; three measles strains; sera from eight SSPE patients, normal individuals, and patients with atypical measles; and hyperimmune rabbit serum to SSPE and measles strains. No evidence was found that unique antigenicity of different M proteins could explain the results. Especially strong evidence was provided by the failure of serum from an SSPE patient to precipitate the M protein of the virus isolated from that patient. Thus, in the sera of SSPE patients there appear to be low levels of antibodies to the M protein in the presence of a hyperimmune response to other viral proteins.

In our view, the most likely explanation for these observations is a defect in the production of functional M protein. Such a defect could also explain other features of SSPE. No infectious virus has been found in SSPE brain or in cultures of brain cells, nor have budding particles been seen in electron microscopic studies of brain or cultured brain cells, although measles virus...
antigens and nucleocapsid are seen (15). The synthesis of a functional M protein is essential for assembly of paramyxovirus particles; it is a structural protein of the viral envelope and serves as the recognition site at the cell membrane for the nucleocapsid during the budding process (16). Thus, a defect in M protein synthesis or function is compatible with the findings in SSPE, in which there is a persistent infection characterized by lack of production of mature virions but the continuing synthesis of other viral proteins leading to a hyperimmune response to them. A defect in the production of functional M protein could arise in several ways: by synthesis of M that is not handled normally in the cell, by diminished synthesis of M; or by synthesis of an aberrant M lacking normal antigenic determinants. Whatever the mechanism, it must be partially host dependent, because virus with a functional M protein antigenically similar to M from measles strains can be rescued from brain cells by cocultivation with permissive cells. This argues against the third possibility, as it seems unlikely that replication in the brain would reversibly affect antigenicity. There is precedent in paramyxoviruses for the synthesis of the M protein and the other viral proteins, but with failure of virus assembly in certain cells—e.g., simian virus 5 in primary chicken embryo fibroblast cells (17). This explanation is attractive, but one could raise the objection that in SSPE antibodies are formed to NP and P, which like M are also internal viral proteins. Thus, an added feature of this hypothesis would be that, in addition to not being incorporated into viral particles, the M protein was less exposed to the immune system, perhaps by binding to intracellular membranes, than are the nucleocapsid-associated proteins (NP and P) which might be released. There is also precedent for a host-dependent decrease in the synthesis of M protein in myxoviruses, in which an abortive infection without production of virus particles is characterized by a deficiency in the synthesis of M (18, 19). The hypothesis that there is diminished synthesis of M, or synthesis of a protein which does not function normally in SSPE brain cells, could be explored in fresh brain tissue from SSPE patients or brain cells in culture by using the immunoprecipitation techniques described here or labeled monospecific antibody to the M protein.

An alternative explanation for the lack of antibody to M in SSPE sera is a specific immune defect in the patients, so that the M protein is not recognized by the immune system. This seems unlikely in view of the hyperimmune response to the other proteins, the lack of convincing evidence for immunological deficiencies in these patients, and the fact that some antibody to M is present in many of these patients. The low levels of antibody that are seen could result from the initial measles virus infection, in which there had to be synthesis of M during the virus replication and spread in the patient to cause the primary disease of measles.

In summary, the most likely course of events appears to be that, after the initial episode of measles, persistent infection is established in the brain, which is characterized by the continued synthesis of other virus proteins leading to a hyperimmune response to them, but by either relatively little synthesis of M or abnormal handling of this protein in the brain cells, resulting in little stimulation of antibodies to this protein.

The absence of clusters of cases of SSPE suggests that there are not circulating in the population specific SSPE strains, infection with which leads to SSPE. This implies that, if SSPE is caused by a mutant of measles virus which involves the M protein, such mutations arise during infection in those individuals who ultimately develop the disease. The rareness of the disease is not incompatible with such an occurrence. The alternative explanation that the observed changes in the biology of M are the result of persistent infection rather than involved in the causation cannot be excluded at this time. Nor can it be excluded that in brain cells in vivo the M protein of measles virus strains in general is handled in an abnormal manner, and it is at the level of the extent of initial infection in the brain and ability to persist for reasons not involving M that SSPE strains are distinguished.

The present findings, although implicating the M protein in the pathogenesis of SSPE, do not support the previous conclusion based on studies with one measles strain that the electrophoretic mobility of the M protein can be used as a marker for SSPE viruses (5), because both SSPE and measles strains varied with respect to the migration of their M proteins, as well as other viral proteins, and there was not a pattern characteristic of SSPE strains. Similarly, we were unable to demonstrate by two-dimensional peptide mapping major differences between the M proteins of the two kinds of viruses. Additional studies with techniques that permit a finer level of discrimination might reveal such differences.

We thank our many colleagues who generously supplied sera. We are particularly indebted to Dr. F. E. Payne who provided the matched SSPE strain and homologous serum, Dr. M. Kaplan for sera from patients with atypical measles and vaccinated children, and Dr. G. Agarsdottir for many SSPE sera. We thank Dr. E. L. Gerseh for CV-1 cells and Mrs. A. Moody for excellent technical assistance. This research was supported by a grant from the Kroc Foundation, and grants from the National Multiple Sclerosis Society (RG 1216-A-2) and from the National Institute of Allergy and Infectious Diseases (AI-05600). W.W.H. is a Rita Allen Foundation Scholar, and R.A.L. is an Irma T. Hirschl Research Career Awardee.