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Measles Virus Matrix Protein Detected by Immune Fluorescence with Monoclonal Antibodies in the Brain of Patients with Subacute Sclerosing Panencephalitis

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Brain materials from four cases of subacute sclerosing panencephalitis were examined by immune fluorescence with monoclonal antibodies against five structural components of measles virus. All five antigens including the matrix component were present in the brain tissues of all cases. A defective Vero cell-associated virus isolate from one of the cases produced all of the structural components except the matrix protein.

Subacute sclerosing panencephalitis (SSPE) is a rare progressive and fatal infection in the central nervous system of children and young adults and is caused by measles virus. This disease usually develops many years after a regular measles infection. SSPE has been the subject of many studies since it is an important example of a persistent infection with an RNA virus that lacks the capacity to integrate into cellular DNA in humans with an intact mature immune defense system (14–16). It has been proposed that defects in the synthesis of the matrix (M) protein may be the source of the restriction of virus maturation in brain cells in SSPE (4–6).

In this study, brain materials from three cases of SSPE (I, II, and III) were collected in Poland. These cases developed characteristic clinical signs of SSPE. Death occurred at 9, 10, and 12 years of age, after a disease course of 16, 18, and 12 months, respectively. Antibody titers estimated by immune fluorescence with persistently infected Lu106 cells (9) were 60,000 to 100,000 in sera and 160 in cerebrospinal fluid. Brain tissue was collected at autopsy 2, 4, and 3 h after the death of the 9-, 10-, and 12-year-old children, respectively. The material was immediately frozen in liquid nitrogen. Pieces of material were transported in dry ice to Stockholm, Sweden, where they were stored at −70°C before being used for immune fluorescence studies. Materials from a fourth SSPE patient (IV) was collected in The Netherlands. This material derived from a 9-year-old girl, with a disease course of about 1 year. This patient died 2 months after the biopsy, and no autopsy was performed. Brain biopsy tissue and a cell-associated virus isolate to which dimethyl sulfoxide was added were stored in liquid nitrogen and analyzed. Results from previous studies, including virus isolation, of material from this patient have been published (8).

Frozen brain sections (thickness, 10 μm) were cut, fixed in cold (−20°C) acetone for 10 min, and stored at less than −20°C until used. Infected Vero cells were fixed and stored in the same way. The preparations were examined by immune fluorescence (7) with diluted (1:50, or in the case of negative results 1:20) mouse ascites fluid containing monoclonal antibodies. Controls included murine ascites fluid not containing measles virus-specific antibodies and sections from noninfected autopsy brain or tissue culture material.

Brain material from the four cases of SSPE were studied by immune fluorescence with two monoclonal antibodies representing two different epitopes on each of the nucleoprotein (NP), polymerase (P), hemagglutinin (H), and fusion (F) components and nine monoclonal antibodies identifying six different epitopes on the M component (10, 12, 13). The frequency of occurrence of infected cells varied in the different materials. In one of the autopsy cases (II, Fig. 1) many thousands of infected small oligodendroglialike cells were seen in the white matter. In the gray matter some neurons with their neuritic extensions were infected. The other autopsy materials contained fewer infected cells, and in the biopsy material only about 20 infected cells could be identified in each section.

The monoclonal antibodies to the NP antigen invariably stained large intranuclear inclusion bodies and granular material in the cytoplasm (Fig. 1a). In contrast, antibodies to the P protein only stained cytoplasmic granules, although with a strong intensity (Fig. 1b). The same difference in staining of cells by monoclonal antibodies against NP and P antigens was previously observed in tissue culture systems (10). Antibodies to the two surface antigens gave positive staining in all four materials (Fig. 1e; staining of F antigen not illustrated); however, whereas the staining of F antigen in all cases was of moderate intensity, the staining of H antigen varied between moderate-strong and weak in different cases (Table 1). Somewhat unexpectedly, the M antigen was readily detected in all four brain materials (Fig. 1c and d). In three cases eight to nine antibodies gave a moderate-strong staining, whereas in case III five antibodies gave only a weak staining and no detectable staining was seen with two antibodies.

As previously described (8), a cell-associated virus isolate was obtained from patient IV. The virus isolation was made by cocultivation of trypsinized brain biopsy material and different cell lines, including Vero cells. Vero cells were used for further passaging of virus for the present studies. The monolayer cultures displayed focal signs of virus replication. Limited syncytia developed (Fig. 2a), but after a few days the infected cells detached. This left a defect in the monolayer (Fig. 2b); however, within a few days this defect was filled out by replication of cells in its periphery.

Immune fluorescence analysis with monoclonal antibodies gave the following results. Antibodies to both NP and P

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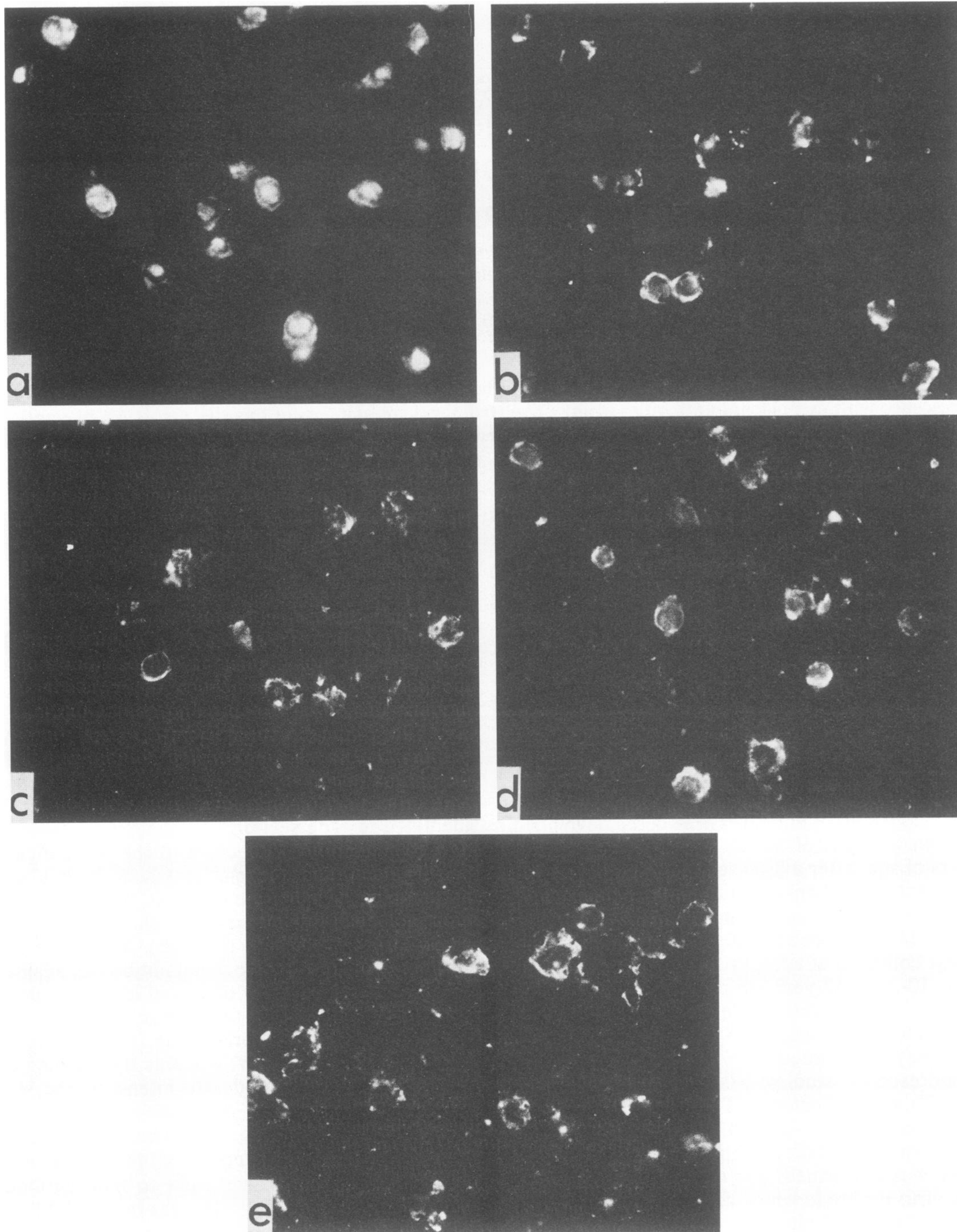

FIG. 1. Immune fluorescence analysis of brain sections from SSPE case II with monoclonal antibodies. Results of staining with monoclonal antibodies against NP (a), P (b), and two different epitopes on M (c and d) and H (e) components. Note the staining of large intranuclear inclusions with antibodies against the nucleocapsid but staining only of cytoplasmic material with antibodies against other structural components.

antigens gave a pronounced staining, but only antibodies to the NP antigen stained intranuclear inclusions. In contrast, H and in particular F antigens were weakly stained. Four different antibodies against each of the surface components were used, and in the case of one antibody against the F antigen no specific staining was detectable. Several experiments were performed with different monoclonal antibodies against the M protein. Antibodies representing all of the hitherto identified six epitopes (12) were used. The results were invariably negative.

The present study shows the occurrence of M antigen in the brain of four patients with SSPE. This finding contrasts with the results of previous studies of SSPE brain material which did not allow detection of any M antigen (5) or in only one case of four found an in vitro translation of M-specific mRNA (1). There are three possible explanations for these

TABLE 1. Summary of reactions in immune fluorescence with monoclonal antibodies of various measles virus component specificity and brain sections of four SSPE cases

Specificity of antibody activity (no. of monoclonal antibodies)	Quality of staining	Intensity of staining with different SSPE cases			
		I	II	III	IV
NP (2)	Large intranuclear and cytoplasmic inclusions	←——— Strong ———→			
P (2)	Only cytoplasmic inclusions	←——— Strong ———→			
M (9)	Only cytoplasmic, but more diffuse than antibodies against the P component	8 moderate-strong, 1 weak	9 moderate-strong	2 moderate, 5 weak, 2 negative	9 moderate-strong
H (2)	Diffuse cytoplasmic with accentuation towards cell periphery	2 moderate-strong	1 moderate, 1 weak	1 moderate, 1 weak	2 weak
F (2)	Similar to antibodies against the H component	←——— Moderate ———→			

divergent observations. The first possibility is the occurrence of either qualitative or quantitative differences between the techniques used. Thus, it could be that the immunoblotting technique used in one previous study (5) had a low capacity to detect M antigen. A second possibility is that there is a variation between virus strain characteristics in different cases of SSPE. It appears probable that such a variation may occur. However, it seems unlikely that all four SSPE cases examined in this study would display an exceptional behavior with regard to M-antigen production. A third explanation for various results in detecting different structural components could be alterations in their synthesis

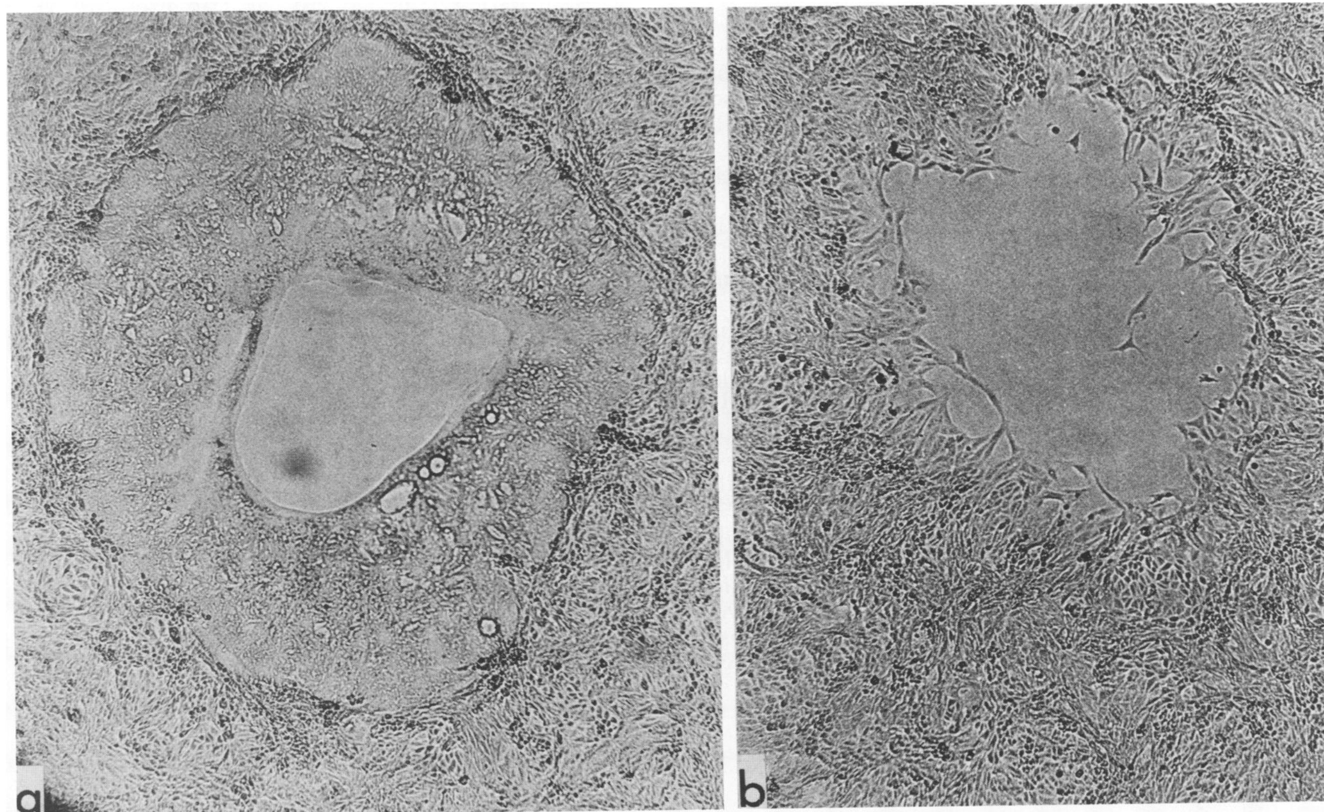

FIG. 2. Infection with the cell-associated virus isolate from case IV in Vero cells. (a) Focal formation of a syncytium; (b) beginning of the healing of the defect in the monolayer after detachment of infected cells.

during different phases of disease. Thus, the antigen expression could be expanded during the acute phase of the disease but then become more restricted during later phases, possibly as a result of immune modulation (3) or immune destruction. There is evidence that the amount of viral antigen in the brain is reduced as the duration of the disease is extended (2). All four cases studied showed a moderately rapid evolution of disease, and an abnormal disease course therefore does not provide a likely explanation for present findings.

As a consequence of the observations in this study, any hypothesis concerning the pathogenesis of SSPE has to consider the occurrence of synthesis of the whole or parts of the M antigen in brain cells at least during some stage of the disease process. The most plausible explanation of events in SSPE pathogenesis may be the following. Wild virus may remain in the brain after the acute infection, possibly under the control of host cell restriction. At some stage, a variant of the virus is selected under the modulating conditions provided by the immune response (3). The nature of the virus causing disease probably varies from one patient to the other. Although in all cases the virus is defective and cannot mature into complete virions in the brain, this does not preclude the occurrence in some cases of an intact virus genome, since occasionally cocultivation experiments allow isolation of lytically replicating virus.

The possibility that the primary maturation defect in virus replication in the brain of SSPE patients concerns the M antigen still remains open. The M protein detected by monoclonal antibodies might be incomplete or for other reasons nonfunctional. However, it is worth noting that there also is variation in the expression of other envelope components, especially the H component. This has been observed under both in vivo and in vitro conditions. Serological studies have revealed not only a poor response of antibodies to the M component but also that the antibody response to the F component is more accentuated than that to the H component (11). Therefore, different alternate mechanisms for emergence of maturation defects should be considered. It is of paramount importance that the virus-infected brain cells cannot be specifically identified by the immune system.

The difference between detectable M-protein synthesis by the same SSPE virus strain in brain biopsy material and in isolated tissue culture by cocultivation is interesting. Some different explanations can be considered. Host cell restriction of M-protein synthesis might occur in Vero cells but not in brain cells. This appears less likely in view of the permissiveness ordinarily shown by Vero cells for measles virus replication. Another possible explanation could be that M antigen is synthesized in both kinds of cells but that the turnover rate is much higher in Vero cells. Perhaps, however, the most likely explanation is a difference in the duration of infection of cells in the brain and in Vero cell cultures. It appears likely that the infection in brain cells persists for a long time, possibly weeks or even months. This is evidenced by the unique accumulation of large quantities of nucleocapsids in cell nuclei. Under these conditions, even small amounts of M antigen resulting from a slow synthesis could accumulate and allow detection by immune fluorescence. In contrast, the amount of M antigen synthesized in Vero cells during 2 to 3 days of incubation may be too low to allow detection. A certain caution in extrapolation of findings made in in vitro systems to the situation in the brain of virus-infected patients therefore may be motivated.

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