A Comparison of Polypeptides in Measles and SSPE Virus Strains

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SUMMARY

The polypeptide patterns of twelve subacute sclerosing panencephalitis (SSPE) and measles viruses have been compared by slab gel electrophoresis. The polypeptide patterns of nine strains of SSPE and measles virus were identical. Differences in the NP protein and the HA protein of the Oddo strain, the P protein of the large plaque variant of the Lec strain of SSPE and in the M protein of the Hu 2 measles strain could not be correlated with biological characteristics such as plaque morphology, origin or haemagglutination properties.

A number of strains of measles virus have been derived from brain tissue of patients suffering from subacute sclerosing panencephalitis (SSPE). It is not known whether SSPE virus represents a distinct strain, which is the aetiological agent for SSPE, or whether the disease is caused by a typical measles virus, which possibly may be modified during the persistent infection in the brain. To date there has been no extensive survey of the biochemical properties of different measles and SSPE viruses and this is probably due to the difficulties in obtaining virus stocks of sufficiently high titres and in purifying the virus free from host proteins (Mountcastle & Choppin, 1977; Rima & Martin, 1979). One preliminary report indicated that the membrane (M) protein of SSPE virus is larger than that of measles virus (Schluederberg et al. 1974), and recently Wechsler & Fields (1978a) have compared the mobility of polypeptides of a number of SSPE virus strains with one measles strain and reported minor differences in the mobility of the M proteins of SSPE virus in infected cells lysates.

In this study we examined the virus polypeptides of several strains of measles virus and SSPE virus to see if there are any major differences relating to the origin of these strains or to variations in biological characteristics.

As reported earlier (Schluederberg & Nakamura, 1967; Gould et al. 1976; Shirodaria et al. 1976) two types of measles virus strains exist. One type (HA) has haemagglutinating activity in the presence and absence of 0.8 M-ammonium sulphate whereas the other type (SDA) agglutinates monkey red blood cells only in the presence of salt. Strains of both types were included in this study. The origins, plaque morphology and salt-dependency of the haemagglutinating activities of some of the strains used here [Edm 1; P9; Hu 1; Hu 2; Schwarz; Lec-LP; Lec-SP; SSPE(1)-LP and SSPE(1)-SP] have been described before (Gould et al. 1976; Shirodaria et al. 1976), and another sample of the Lec SSPE was obtained from Dr V. ter Meulen. The P9 foci variant of P9 forms dense foci rather than syncytia on monolayers of Vero cells (E. A. Gould & S. L. Cosby, unpublished data). The Oddo strain is the UP variant described by Oddo et al. (1961). The Hu 2 strain was isolated from lung tissue of a dysgammaglobulinaemic child that had been vaccinated with the Schwarz vaccine strain. Procedures for labelling the strains, for virus purification, slab gel electrophoresis and autoradiography have been described elsewhere (Rima & Martin, 1979). Variations in the stoichiometry of virus polypeptides were obtained but they appeared to be dependent on the nature of the infection, in particular the presence of defective interfering
material, the m.o.i. and the time of labelling. It proved impossible to standardize these factors over the large range of isolates that we studied. Therefore we compared only the mobility of the polypeptides of the various strains. We have used the Edmonston (Edm 1) strain as a reference for comparison purposes.

In Fig. 1(a, b) and 2(a) the polypeptides of the Edm 1 strain are compared with those of other measles virus strains and Fig. 2(b) shows a comparison between the Edm 1 strain and the SSPE isolates studied. For convenience, we shall summarize the polypeptide composition of measles virus here as it has emerged from recent studies (Mountcastle & Choppin, 1977; Hardwick & Bussell, 1978; Tyrrell & Norrby, 1978; Wechsler & Fields, 1978a, b; Rima & Martin, 1979). Measles virus contains, like other paramyxoviruses, a large L-polypeptide (mol. wt. 180K), a nucleocapsid associated P-polypeptide (mol. wt. 70 K) the nucleocapsid protein NP (mol. wt. 60 K), a membrane or matrix protein M (mol. wt. 37 K), a large glycoprotein HA (mol. wt. 80 K) and a smaller glycoprotein F0 (mol. wt. 60 K). Graves et al. (1978) have shown that this F0-protein, present in infected cells, is cleaved into a non-glycosylated F1-protein (mol. wt. 40 K) and a smaller glycosylated F2-protein (mol. wt. about 15 K).

We shall itemize the similarities and differences in virus polypeptides between the strains as follows:

**L-protein:** no differences in the mobility of this protein were observed.

**HA-protein:** the mobility of the HA polypeptide was the same in most measles and SSPE strains. However, in the Oddo strain (Fig. 2a) it moved slightly faster than that of the Edm 1 strain.

**P-protein:** a variation in the mobility of the P-protein was detected. The P-protein of Lec-P strain migrated more slowly than the P-protein of any other measles strain (Fig. 1a, 2b).

**NP-protein:** the NP-protein of the Oddo strain is slightly larger than that of the other measles viruses. Minor variations in the NP-proteins of various measles virus strains have been described earlier by Mountcastle & Choppin (1977).

**F1-protein:** the amount of F1 protein (40 K) in the various preparations was variable. Nevertheless, we identified this polypeptide in all strains tested and no size differences were observed.

**M-protein:** the M-protein of the Hu 2 strain was larger than that of the other SSPE and measles viruses. The differences observed are of the same order as the differences in the M-protein of the Mun-Ht strain of SSPE virus described by Wechsler & Fields (1978a). The M-protein of the Hu 2 strain remained larger by 1500 to 2000 mol. wt. after seven more tissue culture passages. Our analysis of another recent measles isolate (data not shown) indicates that its M-protein is also larger than in the Edm strain. Further studies on a large number of fresh measles isolates will be required to determine if a large sized M-protein is a characteristic of wild-type measles virus.

The mobilities of the polypeptides of the various strains have been studied under a number of electrophoresis conditions on gels of different concentrations, on gradient gels, including the tris-glycine (Rima & Martin, 1979) and phosphate buffer systems (Bussell et al. 1974; Hardwick & Bussell, 1978) and in the absence and presence of urea. In all cases the same differences in the mobilities were observed. We have also confirmed that any altered polypeptides were virus-induced rather than host contaminants by pulse labelling infected cells with $^{35}$S-methionine throughout the growth cycle as described by Rima & Martin (1979).

Fig. 1(a and b) contain the polypeptide patterns of a number of SDA strains (P9, P9 foci, Hu 1, Schwarz) and of strains containing conventional haemagglutinating activity such as
Edm I and Lec-LP. One might expect differences in haemagglutination properties to be reflected in the large glycoprotein (HA) or possibly in the M-protein. However, no differences in the mobilities of either protein could be correlated with the haemagglutinating properties of these strains. As Breschkin et al. (1977) recently reported that the differences between these two types of strain may be generated by a single mutational event, it is unlikely that they would be detectable in our SDS-PAGE analysis.

The P9 foci strain (Fig. 1b) in infected cells shows a type of c.p.e. very different from that of its parent P9. It forms dense foci instead of syncytia, yet the polypeptide patterns of the P9 foci and P9 virus strains appear to be identical.

As our initial data on SSPE viruses differed markedly from those of Schluederberg et al. (1974) we have investigated in detail a variety of SSPE and other recent isolates and in particular the large and small plaque variants of the Lec and SSPE(1) strains. In Fig. 2(b) the polypeptide patterns of these SSPE strains and the Edm I strains of measles virus are compared. Only the Lec-LP strain had a P-protein of different mobility (see also Fig. 1a) but this could not be correlated with either the origin of the virus (SSPE) or its plaque morphology. More importantly however, no difference between the M-proteins or between any of the other polypeptides were observed. The Lec-SP isolate recently obtained from Dr ter

Fig. 1. Autoradiograms of two 7.5% polyacrylamide gel slabs of 35S-methionine labelled purified virus preparations. Slab gel (a) contains in lane (1) Lec-LP; (2) Edm 1; (3) Hu 2 and (4) Schwarz vaccine virus. Slab gel (b) contains in lane (1) P9 Foci; (2) P9; (3) Edm 1 and (4) Hu 1 virus.
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Fig. 2. Autoradiogram of two 7.5% polyacrylamide gel slabs of *S*-methionine labelled purified virus preparations. Slab gel (a) contains in lane (1) Edm 1 and (2) Oddo. Slab gel (b) contains in lane (1) Lec obtained from Dr ter Meulen; (2) Lec-LP; (3) Lec-SP; (4) Edm 1; (5) SSPE(I)-SP and (6) SSPE(I)-LP.

Meulen had polypeptide patterns similar to the Edm 1 strain. The large plaque variant of the SSPE(1) strain showed contamination by host proteins and the low levels of M- and F1-proteins which are characteristic of virus stocks that are defective in growth and maturation (Rima & Martin, 1979).

Unfortunately, the poor growth of measles virus isolates obtained from SSPE patients makes it impossible to assess polypeptide patterns prior to extensive laboratory adaptation. Therefore differences which were present originally could be lost or differences could appear as is indicated by the differences in polypeptide patterns of the three Lec isolates studied. The Lec-LP strain was the major component in our Lec isolate and it differed in the P-protein from the Lec isolate obtained from Dr ter Meulen. Tyrrell & Norrby (1978) also failed to detect differences between their Lec SSPE strain and Edmonston measles virus. The variations observed in the Lec strain with regard to haemagglutination properties, plaque morphology and polypeptide patterns emphasize the need for caution when considering their relevance to such questions as the aetiology of SSPE. The hypothesis that the causative agent of SSPE is an atypical measles virus is favoured by the previously reported difference of 2000 between the mol. wt. of the M-proteins of measles and SSPE virus.
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(Schluederberg et al. 1974). Recently this idea gained further support from the finding of small differences between the P and M polypeptides of SSPE and those of measles virus (Wechsler & Fields, 1978a). Differences in the mobility of the P-protein could not be correlated with SSPE origin (Wechsler & Fields, 1978a) and were also observed between nuclear and cytoplasmic extracts of measles infected cells (Wechsler & Fields, 1978b). The reported differences in the M-protein are very small, and as Wechsler & Fields (1978a) used only one measles virus strain for comparison, it is difficult to assess whether or not these minor variations are a marker for SSPE viruses. In another report (Hall & ter Meulen, 1976) it was shown that cells infected with SSPE viruses contained about 10% extra mRNA when compared with measles infected cells. In a subsequent report (Hall et al. 1978), the number, size and stoichiometry of the mRNA populations of SSPE and measles virus infected cells revealed only small differences in the size of the smallest mRNAs. However, no differences were found in the mobility of the M proteins although they appear to be antigenically unrelated (Hall et al. 1978).

In this study we have found no significant differences in the size of the M-protein nor in the number and size of the newly induced proteins in pulse-labeling experiments on cells infected by SSPE or measles strains. In fact, the variations we have found between the SSPE isolates studied and the Edm 1 vaccine strain were smaller than the differences between the Edm 1 and the Oddo strain and the recent measles isolate Hu 2.

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