Persistent and Lytic Infections with SSPE Virus: A Comparison of the Synthesis of Virus-specific Polypeptides

(Accepted 29 June 1980)

SUMMARY

The synthesis of virus-specific polypeptides and messenger RNA in cell cultures persistently infected with an isolate of measles virus from a patient with subacute sclerosing panencephalitis (SSPE) has been compared to that found in a lytic infection with the homologous virus. The persistent infection described here was chosen as its biological characteristics reflect those of virus-infected brain cells from SSPE patients. The synthesis of H, N and possibly F protein was seen in both lytic and persistent infections, but the synthesis of M protein was only detected in the lytic infection. However, messenger RNA isolated from either the lytic or persistent infection directed the synthesis in a cell-free translation system of all structural polypeptides, including M, and also three non-structural polypeptides, with mol. wt. of 34000, 30000 and 18000. Messenger RNAs coding for the virus-specific polypeptides were also shown to be polyadenylated. In addition, those polypeptides made in vitro which were antigenically related to the haemagglutinin, demonstrated structural changes after passage through a persistent infection.

Measles virus is a highly contagious and ubiquitous agent causing an acute infection in man. Normally, the defence mechanisms of the host control this infection, and provide a life long immunity. However, a late complication of this infection occasionally develops affecting the central nervous system (CNS) several years after the onset of the acute disease. This CNS involvement, known as subacute sclerosing panencephalitis (SSPE), is characterized by a persistent measles virus infection in brain tissue inducing a chronic disease process which leads to death (ter Meulen et al., 1972; Agnarsdottir, 1977). In this disease, patients show elevated titres of measles-specific antibodies in both serum and cerebrospinal fluid (CSF) specimens (Mehta et al., 1977). These antibodies are directed against the H, N and F structural polypeptides of measles virus with little or no activity against the M polypeptide (Hall et al., 1979; Wechsler et al., 1979; Stephenson & ter Meulen, 1979). Although antibody against M protein is only found in early convalescent sera from acute measles (Machamer et al., 1980), the lack of antibody against M protein in SSPE patients is surprising in view of the humoral hyperimmunity against other measles virus structural proteins and thus may indicate that in SSPE, persistently infected brain cells produce little or no M protein. This interpretation is supported by studies in tissue cultures derived from SSPE brain material where only the H, N and F protein of measles virus could be detected (Hall & Choppin, 1979; Lin & Thormar, 1980; Machamer et al., 1981). Such observations suggest that in these cultures a block in virus expression may occur either at the level of transcription or at the level of translation. In order to test this hypothesis a comparative analysis of such a persistent infection with cells lytically infected with SSPE virus has been carried out and is reported here.

Cells persistently infected with the LEC virus, isolated from an SSPE patient, were initially established as follows. Hamsters were inoculated intracerebrally with LEC virus grown in Vero cells. Brains from animals with a subacute encephalitis were trypsinized and
co-cultivated with Vero cells in Eagle's (minimum essential medium) MEM containing 5% (v/v) foetal calf serum. The surviving cells were cloned and a persistently infected cell line was obtained which was carried in our laboratory for about 200 passages over a period of several years. During the experiments described here, cells from passages 200 to 240 were used and exhibited haemadsorption with monkey red blood cells. Between 50 and 95% of the monolayer contained intracytoplasmic and intranuclear measles-specific antigen, as judged by indirect fluorescent staining with serum from patients with SSPE. However, no infectious virus could be detected in these cultures. Therefore, this persistent infection parallels closely the situation in persistently infected brain cells from SSPE patients (Katz & Koprowski, 1973).

Vero cells, lytically infected with SSPE virus LEC, were labelled with L-[35S]methionine (Amersham/Buchler) between 20 and 22 h post-infection and immune precipitations performed as described previously (Stephenson & ter Meulen, 1979). Uninfected and persistently infected cells were similarly treated.

For messenger RNA isolation, Vero cell monolayers were grown in roller bottles and infected with LEC virus at an m.o.i. of 1 as described previously (Stephenson & ter Meulen, 1979). Cell sheets were harvested by shaking the bottles with sterile glass beads when 95 to 100% of the cells had formed syncytia (20 to 22 h after infection). Uninfected and persistently infected cultures were harvested in a similar manner. Each extraction contained about 5 x 10^8 cells. The cell suspension was then centrifuged for 5 min at 4 °C and 1000 g and the pellet resuspended in 20 ml RSB (10 mM-NaCl, 10 mM-tris-HCl pH 7.4, 1.5 mM-MgCl2). The cell homogenate was disrupted on ice with 30 strokes of a Dounce homogenizer and centrifuged for 5 min at 4 °C and 1000 g. The supernatant was taken and centrifuged again. The second supernatant was made 100 mM with respect to NaCl, 50 mM with respect to sodium acetate (pH 4-6), 2.5 mM with respect to EDTA and 1% (w/v) with respect to SDS. RNA was then extracted from this suspension with phenol as described previously (Stephenson et al., 1977). The ethanol precipitate from this extraction was dried and dissolved in 2 ml adsorption buffer [500 mM-LiCl, 50 mM-tris-HCl pH 7.4, 1 mM-EDTA, 0-5% (w/v) SDS] and poly(A)-containing RNA was purified on an oligo(dT)-cellulose column as described previously (Stephenson et al., 1977). The high salt eluate from this column was precipitated overnight at -20 °C in 3 vol. absolute ethanol and centrifuged for 30 min at 4 °C and 5000 g. The pellet was dried, dissolved in water, adjusted to a concentration of 1 mg/ml and designated ‘pA’. The low salt eluate was made 100 mM with respect to NaCl, precipitated as above, dissolved in water and designated ‘+pA’. RNA was translated in the rabbit reticulocyte lysate prepared as described by Pelham & Jackson (1976) with the modification described by Siddell et al. (1980).

The synthesis of virus-specific polypeptides was detected in both lytically and persistently infected cells by precipitating [35S]methionine-labelled cell lysates with hyperimmune rabbit serum and analysing the product on polyacrylamide gels. Polypeptides corresponding in mobility to the major nucleocapsid protein (N), the second major internal virion polypeptide (M) and the fusion protein (F1) were readily detected in lytically infected cells (Fig. 1a, track 2). In addition, the major external antigen (H) was seen and this protein has been shown to be responsible for the haemagglutinin activity of the virus (Stephenson et al., 1981). The identity of the polypeptide designated 90K is uncertain, but it is thought to correspond to the 70K or ‘p’ species reported by other workers. By analogy with Sendai virus, the polypeptide species designated NC is assumed to be a cleavage product of the N polypeptide (Lamb & Choppin, 1977). As the polypeptides labelled L1 and L2 were found in immune precipitates formed between uninfected cell lysates and measles-specific sera (Fig. 1a, track 1) and could not be detected in persistently infected cultures from hamster brain, they are thought to be Vero cell proteins which have been incorporated into the partially purified virus preparations used to
raise this antisera. The polypeptide labelled ‘50K’ is thought to be either the precursor of the fusion protein or possibly another degradation product of the ‘N’ protein.

When polypeptides from persistently infected cell cultures were analysed only the H, N, 50K and NC virus-specific polypeptides but not the M and the F1 polypeptides could be immunoprecipitated (Fig. 1a, track 3). These results would suggest that the synthesis of at least one major virus polypeptide, namely the M protein, is blocked either at the level of transcription or at the level of translation.

In order to distinguish between transcriptional and translational control in these systems, messenger RNA was extracted and translated in a cell-free system. When total cytoplasmic RNA from either uninfected cells or cells lytically or persistently infected with measles virus were fractionated on an oligo(dT) column, only those RNA species containing sequences of polyadenylic acid [poly(A) sequences] were capable of stimulating a cell-free translation system from rabbit reticulocytes (Fig. 1b, tracks 3, 5 and 7). This observation is similar to
that reported for other members of the paramyxovirus group (Weiss & Bratt, 1974). A major protein band with a mol. wt. of 42 000 was formed when poly(A)-containing messenger RNA from either uninfected or infected cells was added to the cell-free protein-synthesizing system; this was assumed to be actin (AC). Lysates incubated with messenger RNA from either lytic or persistent infections contained major amounts of polypeptides with mol. wt. of 60 000 and 38 000 which correspond in mobility to the N and M polypeptides respectively of the virus (Fig. 1 b, tracks 5 and 7). Similar results have been obtained from lytic infections using other translation systems (Sprague et al., 1979; Niveleau & Wild, 1979) or other persistent infections (Rozenblatt et al., 1979). The virus origin of the 40K polypeptide, seen in persistently infected cells (Fig. 1 b, track 7) but never in uninfected Vero cells, is uncertain as it could not be precipitated from either lytic or persistent infections with virus-specific antisera (Fig. 2 a, track 2 and Fig. 2 b, tracks 3 and 7). The 44K species is thought to correspond to the cleaved N protein (NC) seen in Fig. 1 (a). The origins of the 76/74K, 34K, 30K and 18K are discussed below.

When the products of a reticulocyte lysate, incubated with messenger RNA from lytically infected cells, were precipitated with measles-specific, hyperimmune rabbit sera, nine polypeptides could be seen (Fig. 2 a, track 2 and Fig. 2 b, tracks 1 and 3). Polypeptides with
mol. wt. similar to the virion N polypeptide, its putative cleavage product NC and the M polypeptide were seen. The polypeptide species with an apparent mol. wt. of 50000 (50K) had a similar mobility to the putative precursor of the fusion protein (Fig. 1 a) (Stephenson & ter Meulen, 1979; Graves, 1981) and it is thought that this represents the non-glycosylated version of this polypeptide. However, at present we cannot exclude the possibility that this is another cleavage product of the N protein. The doublet species with apparent mol. wt. of 74000 and 76000 have been shown to be related to the haemagglutinin protein of the virion as both species are precipitated with monoclonal antibodies raised against the H protein of the virus (Stephenson et al., 1981). These polypeptides could therefore represent non-glycosylated precursors of the H protein. The polypeptide species with mol. wt. of 18000 (Fig. 1 b, track 5 and Fig. 2 a, track 1) is thought to be virus-specific as it was precipitated with antisera raised against purified virus (Fig. 2 a, track 2) and was not seen in uninfected cells (Fig. 1 b, track 3). Although this species has not been reported in purified virus particles a protein with a similar mol. wt. has been reported in lytically infected cells, labelled in vivo, by other workers (Vainionpää et al., 1978; Tyrrell & Norrby, 1978) and it has been suggested by some as a candidate for part of the fusion protein. However, as specific proteolytic cleavage does not normally take place in vitro, and the F (40K) polypeptide was not seen in immune precipitates of our translations, the 18K polypeptide reported here would appear to be a primary gene product. The species with mol. wt. of 34000 and 30000 (Fig. 1 b, track 5 and Fig. 2 a, track 1) are also thought to be virus-specific as they were not seen in uninfected cells (Fig. 1 b, track 3) but were precipitated with virus-specific antisera (Fig. 2 a, track 2 and Fig. 2 b, tracks 1 and 3). Neither the 18K nor the 34K/30K species are thought to be degradation products from other virus proteins as their messenger RNAs sediment at a slower rate than do those of the other major virus proteins (data not shown). However, whether they represent translation products of poly(A)-containing defective RNA, premature termination products in vitro, or they are genuine virus-specific polypeptides whose translation is suppressed in vitro, is not known at present.

When the products from a cell-free system incubated with messenger RNA from a persistent infection were precipitated with hyperimmune rabbit serum, the polypeptide profile was broadly similar to that seen in a lytic infection (Fig. 2 b, track 7). However, the overall level of virus-specific polypeptide synthesis appeared to be lower when the same total amount of RNA from the persistent infection was translated compared to RNA from the lytic infection, and this may account for the apparent absence of the 50K and NC polypeptides in the translation product of RNA from the persistent infection. Notably, the synthesis of the M protein could be demonstrated in the translation products of mRNA from the persistent infection. The only other observable difference between the translation of RNA from lytic and persistent infections is that whereas in the lytic infection the species related to the H polypeptide appeared as a doublet (Fig. 2 b, tracks 1 and 3), in the persistent infection only the smaller species, i.e. the 74K component was seen (Fig. 2 b, track 7). However, as in the case in the lytic infection this species is also precipitated by monoclonal antibodies raised against the haemagglutinin protein from purified virus (Stephenson et al., 1981). The significance of this observation is not known at present as the H protein found on the surface of persistently infected cells appeared to be functionally unimpaired, as judged by the presence of cell-surface haemadsorption.

In conclusion, we have shown that whereas in a lytic infection with SSPE virus all major structural proteins were easily detected, in the persistent infection studied here only the H, N and maybe the F protein could be readily found. However, when mRNA from both persistent and lytic infections are analysed by in vitro translation, messengers for the H, N and M structural proteins as well as three non-structural polypeptides were detectable. Although these data appear to suggest a block at the level of translation for at least one virus
polypeptide, i.e. M, the methods used to detect \textit{in vivo} protein synthesis in persistent infections may not be sensitive enough to permit such an interpretation. In addition, we have found that when mRNAs from a persistent infection were translated \textit{in vitro}, the haemagglutinin protein synthesized appeared to show some structural differences from similar molecules produced by mRNA extracted from a lytic infection. It is conceivable that similar changes in the H polypeptide occur during SSPE. Furthermore, since M protein serves as a recognition site for the nucleocapsid at the cell membrane during the budding process of paramyxovirus (McSharry \textit{et al.}, 1971) its absence, or a defect in its synthesis, would inhibit assembly of SSPE virus. The observation in SSPE of brain cells filled with nucleocapsids without observable budding processes or infectious virus is compatible with such a defect of M protein synthesis.

We would like to thank Magdalene Pföhrer and Andrea Barthel for excellent technical assistance. This work was supported by the Deutsche Forschungsgemeinschaft. We thank Helga Kriesinger and Jane Brett for typing the manuscript.

\textit{Institut für Virologie und Immunbiologie}
\textit{Versbacher Strasse 7}
\textit{8700 Würzburg, Federal Republic of Germany}

\textbf{JOHN R. STEPHENSON†}
\textbf{STUART G. SIDDELL}
\textbf{VOLKER TER MEULEN*}

† Present address: Division of Vaccine Research and Production, Centre for Applied Microbiological Research, Porton Down, Salisbury, Wiltshire, U.K.

\section*{REFERENCES}


*(Received 25 March 1981)*