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Subacute Sclerosing Panencephalitis: Are Antigenic Changes Involved in Measles Virus Persistence?

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INTRODUCTION

Subacute sclerosing panencephalitis (SSPE) is a rare, fatal complication of measles virus infection arising years after acute measles. During this chronic disease process, characteristic measles virus inclusions are present in the cells of the central nervous system (CNS) (Freeman et al., 1967; Connolly et al., 1967; ter Meulen et al., 1967, 1969), and the patients display an elevated serum antibody titer to all measles virus polypeptides. This response does not include anti-matrix protein (M) activity (Hall and Choppin, 1979; Wechsler et al., 1979; Stephenson and ter Meulen, 1979). Where this response can be detected, it is consistent with antibody remaining from the previous acute infection (Hall and Choppin, 1979). A similar pattern is observed in the CSF, where antibodies produced also lack an anti-matrix protein activity. In the CNS, however, the immunoglobulins synthesized are oligoclonally restricted and probably, therefore, secreted from lymphocyte clones which have invaded this compartment. The epidemiology, immunology and current knowledge of the measles virus involved have recently been reviewed (ter Meulen et al., 1983). During SSPE, infectious virus is not present either in the CNS or other tissues, but it has proved possible, in some cases, to rescue a measles-like virus by cocultivation techniques (reviewed by Agnarsdottir, 1977). These viruses may differ from measles virus, and from each other, but no single, stable property differentiates them. In fact, differences between various strains of measles virus are of a similar magnitude to those observed between SSPE and measles viruses (Agnarsdottir, 1977; Fraser and Martin, 1978). In the experiments reported here, we have attempted to differentiate these agents further using monoclonal antibodies raised against purified measles virus Edmonston. In no case is an isolate of measles virus available from the acute phase of infection preceding the develoment of SSPE by the same patient. We have attempted to model this event in vitro by using a tissue culture of Vero cells persistently infected with an SSPE virus "Lec", and to compare this system to other carrier cultures. These in vitro persistent infections differ from any in vivo persistence since they are maintained in the absence of an immune response and may therefore provide an insight into the importance of that phenomenon in the development of SSPE.

MATERIALS AND METHODS

Measles virus Edmonston was grown in Vero or CV-1 cells as described in the text. The Lec SSPE virus was isolated by cocultivation techniques from the brain of a child with SSPE

(Barbanti-Brodano et al., 1970), and a persistent infection (Lec PI) was established in Vero cells as described (ter Meulen et al., 1981). This persistent infection has now been maintained in our laboratory for over two years.

Immune precipitation of infected cell polypeptides was performed using cell [^{35}S]methionine-labeled lysates prepared at 16 h p.i. (Edmonston), 24 h p.i. (Lec lytic) or 16 h after cell passage (Lec PI). Lysate preparation and immunoprecipitation were carried out in RIPA buffer (10 mM Tris-HCl, pH 7.4, 0.15 M NaCl containing 0.1 % SDS, 1 % Triton X-100 and 1 % sodium deoxycholate as described by Lamb et al. (1978). Immunoprecipitated proteins were analyzed on 10 % polyacrylamide gels using the method of Laemmli (1970) and visualized by fluorography (Bonner and Laskey, 1974). Messenger RNA was isolated from cellular cytoplasmic fractions and selected by oligo dT chromatography (Barrett et al., 1979). mRNA was translated in a reticulocyte lysate system kindly provided by Dr. S. Siddell. This was prepared as described by Pelham and Jackson (1976) with modifications described by Siddell et al. (1980). The preparation of monoclonal antibodies and the radioimmuno-binding assay has already been described (ter Meulen et al., 1983). Serological assays, haemagglutination inhibition (HAI), neutralization (NT) and haemolysin inhibition (HLI) tests were carried out as described (Norrby and Gollmar, 1975).

RESULTS

In our laboratory we have prepared some 75 hybridoma antibodies. Of these, 21 were found to immunoprecipitate the H-polypeptide, 1 the nucleocapsid, and 2 the matrix protein of measles virus Edmonston. The remainder were not positive by this technique, even though all bound to high titers in a radioimmunoassay. The biological properties of the anti-H-hybridomas have been tested, and on this basis, these antibodies were divided into 5 groups (ter Meulen et al., 1981). These groups are summarized below (Table I). This type of analysis has

TABLE I

PROPERTIES OF MEASLES HYBRIDOMA MYELOMA ANTIBODIES

All hybridoma failed to inhibit the haemolysin reaction. No antibodies were obtained with high neutralization titers (NT) but undetectable haemagglutination inhibition (HI) titers.

Group	*Example*	*Properties*
1	173	No detectable HI or NT
2	298	HI-positive, no detectable NT
3	585, 26	HI and NT activities similar
4	323	HI > NT. Both activities easily detectable
5	155	HI < NT. Both activities easily detectable

provided the first evidence that a given antigenic determinant on the H-polypeptide could be altered in functional significance between measles and SSPE virus strains. That is, the site for a given antibody may still be present, but the antibody–antigen combination no longer produces the same biological effect (ter Meulen et al., 1981). Preliminary data from direct competitive

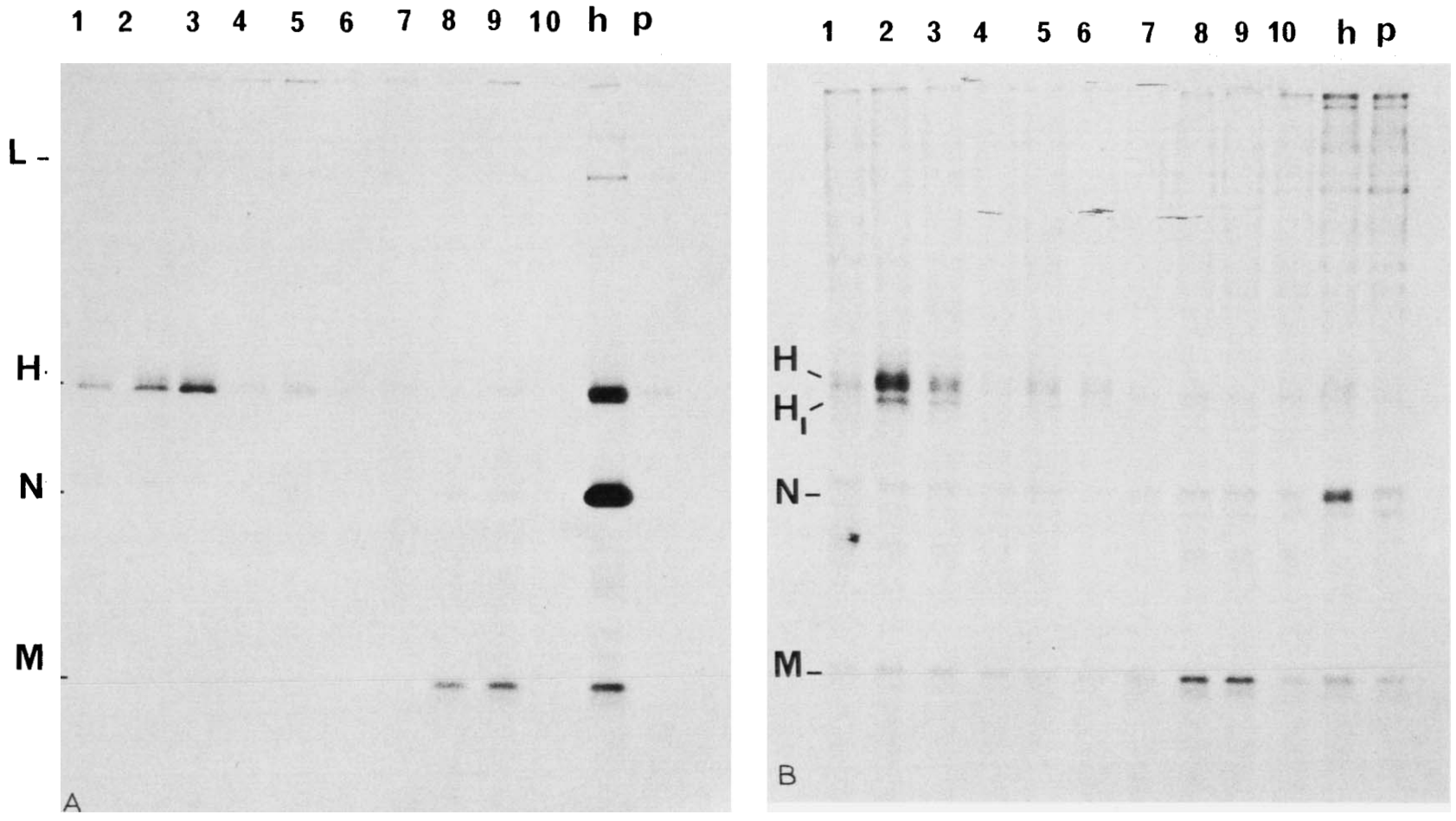

Fig. 1. A: use of hybridoma-antibodies to immunoprecipitate polypeptides from Lec virus-infected Vero cells. Immunoprecipitation was performed in 50 μl vols. at 4° C using 1 μl of undiluted rabbit serum or 5 μl of ascites fluid diluted 1 : 10. Immune complexes were precipitated after 30 min, using staphylococcal protein A coupled to sepharose beads and armed with rabbit anti-mouse immunoglobulins (Dako). h, rabbit hyperimmune serum, raised against purified SSPE virus Lec; p, preimmune rabbit serum; lanes 1–6, anti-H hybridoma antibodies, (nos. 173, 155, 585, 298, 32a, 26); lane 7, anti-N hybridoma antibody (no. 2273); lanes 8 and 9, anti-M hybridoma antibody (no. 128 and 263); lane 10, non measles-virus directed hybridoma antibody. B: proteins immunoprecipitated from Lec PI cell lysates. Method and hybridoma antibody used as in A.

binding experiments indicate that these 5 groups occupy at least 3 partially overlapping sites on the H-protein molecule. The two anti-matrix protein monoclonals (263 and 128) appear to bind at distinct sites on this protein and their binding is not mutually exclusive. Further characterization of the N-polypeptide awaits the isolation of more monoclonal antibodies. This panel of antibodies was used in a comparison of Lec PI cells and Vero cells lytically infected with Lec virus by immunoprecipitation (Fig. 1).

Both anti-M-antibodies were found to immunoprecipitate M-protein from lytic Lec infections. This protein was immunoprecipitated as a double band and this was also observed in some measles virus Edmonston infections. It seems, therefore, that M-protein may exist in two related forms. Recently, Rima et al. (1981) have provided evidence that a cleavage product of the measles virus P-protein also runs in this area. These two hybridoma antibodies also precipitated M protein from Lec PI cell lysates. Owing to the lower level of virus polypeptide expression in this cell line, a much higher background was obtained, but reference to the control monoclonal antibody, raised against a totally unrelated antigen, shows this precipitation to be clearly specific. The anti-nucleocapsid antibody did not react with N protein of the parent Lec virus, nor with that formed in persistence, so that we are unable to draw any conclusions regarding this protein. The anti-haemagglutinin hybridoma antibodies revealed an interesting effect. Only 4 of the 6 antibodies used were clearly positive in the immune precipitation reaction: 173, 155, 585 and 32, when tested with antigens produced in lytic Lec virus infection. These same antibodies also precipitated H protein from Lec PI cell lysates. In this case, however, the monospecific antibodies precipitated the H polypeptide as a double band, H and H_1. Currently, peptide mapping studies are underway to clearly establish the relationship between these polypeptides. Other hybridoma antibodies, namely 298 and 26, were borderline in this technique. In view of the high background, it is difficult to be definitive on this point. Monoclonal antibodies which failed to definitively immunoprecipitate this protein from Lec virus-infected cell lysates, were used in immunofluorescence tests. This provided firm evidence for antigenic change during persistency, since 2 antibodies, 131 and 132, failed to bind to Lec PI cells whilst still combining well with antigens present in cells lytically infected with the Lec parent virus (Table II). This test is more sensitive than immunoprecipitation because a firm antibody–antigen combination is not required.

TABLE II

ANTIGENIC VARIATION IN MEASLES H-POLYPEPTIDE DURING PERSISTENT INFECTION

Immunofluorescence assay using infected tissue culture cells.

Clone number	*Edmonston*	*Lytic Lec*	*Lec PI*
585	+	+	+
131	+	+	–
152	+	+	–
SSPE serum	+	+	+

Thus it may be concluded that antigenic changes have arisen in the course of a persistent infection. These may be related to the double band observed in the specific immunoprecipitation of H-polypeptide from Lec PI cells. Whether this latter effect was due to differences in polypeptide synthesis, modification or turnover was unclear. For this reason, and also in an

attempt to decrease the non-specifically precipitating background, this phenomenon was examined by immunoprecipitation of polypeptides formed in vitro. mRNA was extracted from Lec PI cells or from Vero cells lytically infected with Lec virus. In addition, mRNA was prepared from uninfected cells and from two other measles virus carrier cell lines. The N-1 cell line was produced by the cocultivation technique from SSPE brain material (Doi et al., 1972). The agent carried in these cells is exclusively cell-associated and infectious virus has never been detected. The carrier Lu106 cell line was originally established by Norrby (1967) and does shed infectious virus. The polypeptides detected by this procedure are shown in Fig. 2.

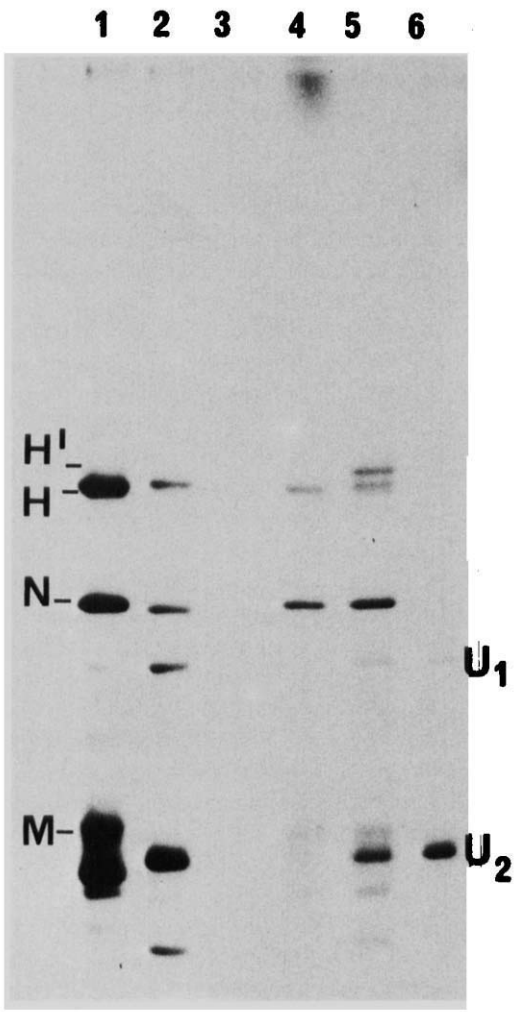

Fig. 2. In vitro translation of mRNA. mRNA was translated in a reticulocyte lysate cell-free system and immunoprecipitated using hyperimmune rabbit serum raised against SSPE virus Lec. The two prominent bands U_1 and U_2 in lane 6 presumably reflect contaminating host-cell polypeptides in this virus preparation. mRNA from: lane 1, carrier Lu cells; lane 2, N-1 cells (SSPE agent); lane 3, no mRNA added; lane 4, Lec PI cells; lane 5, Vero cells lytically infected with SSPE virus Lec; lane 6, uninfected cells. Bands were assigned by reference to a hyperimmune serum precipitation of an infected cell lysate.

Two polypeptides are immunoprecipitated specifically from the translation products of uninfected cells (Fig. 2, track 1) U_1 and U_2. The translation system itself has no immunoprecipitating protein product, and in the absence of added mRNA no products are detected (Fig. 2, track 4). A comparison of proteins formed by the Lec P1 and lytic Lec infected cell mRNA shows that 2 bands are detectable in the H protein region (H and H^1), when mRNA from a lytic infection is used but not when Lec PI cell mRNA is translated. H protein precipitated from Lec virus-infected cell lysates was observed to comigrate with the larger component of the H peptide doublet detected in Lec PI cells (H in Fig. 1). The in vitro translation product of mRNA from Lec PI cells co-migrated with the lower band (H) formed by mRNA extracted from Vero cells lytically infected with SSPE virus Lec. This band may therefore represent the precursor of the fully modified H protein observed in Fig. 1. Since no smaller product was detected amongst the in vitro translation products of Lec PI cells, the split H band (H and H_1) in Fig. 1, may represent post-translational modification of polypeptide H in Fig. 2. This modification either does not occur in lytically infected cells, or cannot be detected in extracts prepared under these conditions.

The higher molecular weight protein H^l in Fig. 2 is at present not understood. It is possible that this product is normally rapidly degraded or modified so that it migrates in a different position. The absence of such a protein in the translation products of mRNA from Lec PI cells, however, is surprising, and suggests a difference in virus expression between the lytic and persistent infections examined. This might be due to the phase of virus replication in which the Lec PI cells are fixed. If this stage is normally short-lived in a lytic infection, then it may be difficult to detect by translation of total cellular mRNA, or by prolonged labeling of cellular proteins. The relevance of these apparent differences in virus expression to antigenic differences between lytic and persistent infections observed in Table II is at present unclear.

Analysis of the M-protein is more difficult since the host band U2 runs close to virus M. The position of M was determined from a lytically infected Vero cell extract. This protein was readily detected among the translation products of virus-releasing carrier Lu cell mRNA. It was present also in the Lec PI protein product, but no protein can be detected in this position in the non-virus yielding N-1 cell line. In a previous communication, we were unable to detect Lec virus M protein in Lec PI cells, but noted this product among the proteins formed in vitro. It would seem that this result can be explained by the high background observed in Fig. 1B, a problem which has now been overcome by the use of anti-M-monoclonal antibodies. Consequently, there is no reason to postulate translational control of M protein in the Lec PI cell system. We are currently searching for mRNA for this protein in the N-1 cell line, using a cloned cDNA copy of the Edmonston M-protein message, and for the protein itself using monoclonal antibodies. The other small bands detected in Fig. 2 are thought to be breakdown products from the nucelocapsid protein.

DISCUSSION

In this report we have observed the occurrence of antigenic change during SSPE virus persistence. This was observed as the loss of ability to bind 2 monoclonal antibodies, and evidence for an alteration in virus expression has been gathered. It seems clear that a rapid mutational drift may occur during virus-persistence in vitro. Holland et al. (1979) reported that a continual evolution in virus-specific RNA was demonstrable during vesicular stomatitis virus persistence. Most of these changes were manifested in the production of small plaque, temperature-sensitive mutants. This is to be expected since it may be supposed that the effect of

most amino acid substitutions would be to destabilize a particular protein, and relatively few would lead to complete loss of function. A similar situation presumably exists during morbillivirus persistence. We have demonstrated changes in H-protein antigenicity during SSPE virus persistence in the absence of antibody selection. Furthermore, the production of small plaque, or ts mutants during measles virus persistence is already well documented (ter Meulen et al., 1973; Haspel et al., 1973; Gould and Linton, 1975; Ju et al., 1978). Moreover, the ts mutants fail to complement each other, and ts (+) revertants retain their small plaque phenotype (Haspel et al., 1973). This suggests each virus had accumulated a number of different mutations. The observation that the measles virus released may alter with time of persistence further supports this concept (Rustigian et al. 1966; Wechsler et al., 1979; Wild et al., 1981).

During measles virus persistence in vivo it is possible that a competent host immune response might drive this mutation such that the virus remained "one-step ahead" of the host. Viruses are able to mutate easily in response to monoclonal antibodies: this is true of influenza (Laver et al., 1979), rabies (Wiktor and Koprowski, 1980), and also of measles virus (Birrer et al., 1981). It is clearly not quite so easy, however, to evade a polyvalent immune response such as encountered in vivo. It is important in this context to emphasize that SSPE viruses are neutralized by circulating antibodies, even if they are not quite so sensitive as measles viruses (Payne and Baublis, 1973). This could lead to conditions of partial neutralization and it is conceivable that a mutant virus might then be able to establish persistence more easily.

A second role of antibody could be modulation of antigen expression. Antibody has been shown to directly influence virus antigen expression, both at the cell surface and intracellularly (Joseph and Oldstone, 1975; Gould and Almeida, 1977; Fujinami and Oldstone, 1979). One of the first casualties of this process seems to be the expression of virus M-protein. Some evidence favours this event in vivo. It had been observed that after hamsters were inoculated with SSPE virus, infectious virus could at first be recovered, but gradually became more cell-associated. The time required for this process could be lengthened by inoculation of anti-lymphocyte serum or thymectomy (Byington and Johnson, 1975; Johnson et al., 1975). Following intracerebral inoculation of hamsters, the expression of M protein was found to gradually decrease, whilst that of N remained high (Johnson et al., 1981). Finally, SSPE patients display a low or absent immune response to M protein, suggesting that this protein is no longer available to the immune system as an antigen.

No M protein can be detected in the SSPE N-1 cell line and it is impossible to rescue virus from this cell line or from a similar line isolated by Thormar et al. (1978). In many other cases attempts to isolate a virus from SSPE autopsy material have failed, whereas in other instances co-cultivation techniques have led to M-protein production and virus release. Thus it is possible that the mechanism of measles virus persistence may differ from one individual to another, or within different areas of the same brain.

SUMMARY

SSPE virus "Lec" was used to establish a persistent infection in Vero cells. Late passages of this culture (225), which shed no detectable infectious virus, were compared to lytic "Lec" virus infections, using monoclonal antibodies in immune precipitation and immune fluorescence reactions. The data indicate that the haemagglutinin molecule (H) has undergone antigenic change during virus persistence in vitro. Antigenic change in the matrix (M) protein was not detected. The Lec persistent infection was compared to carrier cultures of measles virus (Carrier Lu 106 cells), and an SSPE agent (N-1), by in vitro translation of the extracted

mRNA. The relevance of these observations to the mechanism of virus persistence and SSPE is discussed.

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