Characterization of Measles Virus-Specific Proteins Synthesized In Vivo and In Vitro from Acutely and Persistently Infected Cells

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Measles virus protein synthesis has been analyzed in acutely and persistently infected cells. To assess the role of measles in subacute sclerosing panencephalitis (SSPE), measles viral proteins synthesized in vivo or in vitro were tested for reactivity with serum from a guinea pig(s) immunized with measles virus and sera from patients with SSPE. Guinea pig antimeasles virus serum immunoprecipitates the viral polypeptides of 78,000 molecular weight (glycosylated [G]), 70,000 molecular weight (phosphorylated [P]), 60,000 molecular weight (nucleocapsid [N]), and 35,000 molecular weight (matrix [M]) from cells acutely infected with measles virus as well as from chronically infected cells, but in the latter case, immunoprecipitated M protein has a reduced electrophoretic migration. Sera of SSPE patients immunoprecipitated all but the G protein in acutely infected cells and only the P and N proteins from chronically infected cells. In immunoprecipitates of viral polypeptides synthesized in a reticulocyte cell-free translation system, in response to mRNA from acutely or persistently infected cells, the 78,000-molecular-weight form of the G protein was not detected among the cell-free products of either mRNA. Guinea pig antimeasles virus serum immunoprecipitated P, N, and M polypeptides from the products of either form of mRNA, whereas SSPE serum immunoprecipitated the P and N polypeptides but not the M polypeptide. The differences in immunoreactivity of the antimeasles virus antiserum and the SSPE serum are discussed in terms of possible modifications of measles virus proteins in SSPE.

Considerable evidence implicates measles or a closely related virus in the rare degenerative neurological disease subacute sclerosing panencephalitis (SSPE). This eventually fatal disease occurs in children several years after measles infection: it is marked by gross cortical deterioration and loss of function. The sera and cerebral spinal fluid of SSPE patients contain high titers of measles antibodies (4), and paramyxovirus nucleocapsids have been identified in brain tissue (16). Infectious measles-like virus has been recovered by cocultivation of SSPE brain biopsy material with susceptible cells (7, 11). It has been suggested that SSPE results from a persistent measles infection in which chronically infected cells support virus production for extended periods of time, without cytopathic effects or cell lysis. Thus, cell lines of persistently infected measles virus (14, 15) can serve as a model system to study virus expression and its relationship to SSPE.

We have compared the in vivo and in vitro synthesis of measles virus proteins in acutely infected (CV-1) and persistently infected (HeLa)

cells. Use of immunoprecipitation techniques has allowed us to isolate and characterize measles virus-specific proteins, despite the relatively high background of cellular protein synthesis in infected cells. We have analyzed the viral polypeptides synthesized in cell-free, protein-synthesizing systems in response to mRNA from acutely or persistently infected cells and compared the immunoreactivity of specific measles polypeptides with antimeasles virus serum and sera from SSPE patients. Differences in the viral proteins synthesized in acute and chronic infection and in their immunoreactivity with viral or SSPE antisera are discussed.

MATERIALS AND METHODS

Isolation of nucleocapsids. Monolayers of measles virus-infected cells were washed briefly with distilled water and scraped into a small volume of distilled water (5×10^6 to 10×10^6 cells per ml). The cells were homogenized and centrifuged at $2,000 \times g$ for 10 min at 4°C. Triton X-100 was added to the supernatant to a final concentration of 2%, and nucleocapsids were isolated by CsCl gradient centrifugation (9). Cytoplas-

mic extracts were layered onto a discontinuous gradient containing 2.5 ml of 40% (wt/wt) CsCl, 5 ml of 30% (wt/wt) CsCl, and 2.5 ml of 25% (wt/wt) CsCl. The gradients were centrifuged at 23,000 rpm for 2.5 h at 4°C, using a Beckman L5-65 ultracentrifuge with an SW27.1 rotor. The visible nucleocapsid band in the 30% CsCl region was collected.

Cells and viruses. The CV-1 line of African green monkey kidney cells was obtained from Flow Laboratories, Rockville, Md. The cells were grown in Eagle medium supplemented with 10% calf serum. HeLa K11-HG (Cl-11) and K11A-HG-1 (Cl-11A) cell lines persistently infected with measles virus were obtained from R. Rustigian. Cl-11 is a carrier culture which yields low levels of infectious virus, whereas the persistently infected Cl-11A is a "nonyielder" line derived from Cl-11 after extensive serial passage in the presence of antisera to measles virus (15).

The Edmonston strain of measles virus, obtained from B. Fields, had been plaque-purified twice in monkey CV-1 cells. A stock viral suspension (2×10^5 to 6×10^6 PFU/ml) having a marked cytopathic effect was obtained from the pooled fluids of infected cultures.

Labeling of cytoplasmic proteins. Measles virus cytoplasmic proteins were labeled 20 h postinfection with [35 S]methionine (10 to 20 μ Ci; Amersham Corp.) for 1 to 6 h in Dulbecco-modified Eagle medium lacking methionine. After labeling, the cells were washed with reticulocyte standard buffer containing 10 mM NaCl, 10 mM Tris-hydrochloride (pH 7.4), and 0.15 mM MgCl_2 . Cytoplasm was obtained by lysis in reticulocyte standard buffer containing 0.5% Nonidet P-40. Nuclei were removed by centrifugation at $2,000 \times g$ for 10 min at 4°C.

Polyacrylamide gels. Sodium dodecyl sulfate (SDS)-10% polyacrylamide gels were prepared according to Laemmli (8). The gels were run for approximately 2 h at 150 V (constant voltage), stained with Coomassie brilliant blue, dried, and either autoradiographed or fluorographed (2).

Peptide mapping by limited proteolysis. The procedure of Cleveland et al. (3) was used for peptide mapping. The proteins labeled with [35 S]methionine were separated by electrophoresis on 10% acrylamide gels containing SDS. Gels were stained, dried, and autoradiographed. Bands were excised, and the proteins were eluted by electrophoresis overnight into dialysis bags at a constant voltage of 100 V. After exhaustive dialysis against water for 3 days, the samples were lyophilized and redissolved in buffer containing 0.15 M Tris-hydrochloride (pH 6.8), 10% glycerol, and 0.2% SDS to a final concentration of 500 to 1,000 cpm/ μ l. To 20 μ l of this solution, heated at 100°C for 2 min, was added 2 μ l of an aqueous solution of α -chymotrypsin (1 mg/ml). After incubation at 37°C, 5 μ l (total) of 10% SDS, 100 mM β -mercaptoethanol, and 0.1% bromophenol blue was added, and the sample was applied to a 15% SDS-polyacrylamide gel and subjected to electrophoresis for 4 h at 150 V.

Preparation of polyadenylic acid-containing RNA. Cytoplasmic RNA was extracted from 10 cultures of CV-1 cells (2×10^7 cells per culture) 24 h after infection with plaque-purified measles virus at 2 to 4 PFU/cell (13). This method involves several succes-

sive phenol-chloroform-isoamyl alcohol extractions followed by LiCl precipitation. RNA was then subjected to oligodeoxythymidylic acid-cellulose chromatography (1).

Reticulocyte lysates. Reticulocyte lysates from rabbits were prepared by the method of Pelham and Jackson (12). Micrococcal nuclease treatment of the lysate was carried out at a concentration of 20 μ g/ml at 20°C for 20 min. Protein synthesis assays contained 0.015 M creatine phosphate, 90 mM potassium chloride, 125 mM each amino acid except methionine, 0.5 mM magnesium acetate, and 0.1 mg of creatine kinase per ml. A 13.5- μ Ci amount of [35 S]methionine (800 to 1,000 Ci/mmol) and varying quantities of mRNA were added per 25 μ l of reaction mixture. Incubations (at 30°C for 2 h) were terminated by freezing at -20°C.

Immunoprecipitation. Measles virus-specific proteins were immunoprecipitated from cell-free products (0.1 ml) or [35 S]methionine-labeled extracts of measles virus-infected CV-1 cells. Cellular or cell-free products were centrifuged at $12,000 \times g$ for 10 min, and the supernatant solution was incubated with 10 μ l of guinea pig antimeasles virus serum or serum from patients with SSPE for 10 min at 37°C, followed by a second incubation for 1 h at 4°C. Rabbit anti-guinea pig serum or human gamma globulin (0.15 ml), was added; the mixture was left overnight at 4°C, then diluted to 1.5 ml with cold phosphate-buffered saline, and centrifuged at $3,000 \times g$ for 15 min at room temperature. The pellet was washed by centrifugation four times with 1.5 ml of cold phosphate-buffered saline. The immunoprecipitates were solubilized in 50 μ l of electrophoresis buffer (10% [vol/vol] glycerol, 60 mM Tris-hydrochloride [pH 6.8], 2% [wt/wt] SDS), and 5- to 10- μ l portions were analyzed on SDS-polyacrylamide gels.

RESULTS

Measles virus proteins in infected CV-1 cells. (i) Immunoprecipitation. Although there have been reports that measles virus proteins are localized in the nucleus as well as in the cytoplasm (19), analysis of the subcellular distributions of viral proteins in lytic infection revealed that the bulk of these proteins are cytoplasmic in origin (unpublished observations). Cytoplasmic extracts of [35 S]methionine-labeled uninfected monkey cells (CV-1 line) and monkey cells acutely infected by measles virus were analyzed by SDS-polyacrylamide gel electrophoresis.

Several new polypeptides appeared in the cytoplasm of infected cells, with molecular weights estimated as 78,000, 70,000, 60,000, 40,000, and 35,000. This is consistent with previously reported data. The 78,000-, 70,000-, 60,000-, and 35,000-molecular-weight polypeptides, respectively, are probably the G (glycosylated), P (phosphorylated), N (nucleocapsid), and M (matrix) proteins previously described (9, 17, 19).

The major polypeptides are immunoprecipitated from [35 S]methionine-labeled infected cell

extracts (Fig. 1), with molecular weights estimated as 78,000, 70,000, 60,000 and 35,000; again, they are probably the previously designated G, P, N, and M proteins. None of these were obtained upon reaction of uninfected cells with guinea pig antimeasles serum. In the infected cells, a group of polypeptides with molecular weights ranging from 43,000 to 45,000 are precipitated; the largest of these was also found in considerable amounts in uninfected cells. However, their presence in the immunoprecipitate has not been consistently observed, and this group is still under investigation. The novel 40,000-molecular-weight polypeptide seen in infected cells was not immunoprecipitated with guinea pig antimeasles serum, suggesting that this is a nonstructural viral protein, a non-immunoreactive structural viral protein, or a cellular protein whose synthesis is induced after viral infection.

The serum from patients with SSPE contains antibodies which react with the measles virus-infected cells, as can be shown by fluorescent-antibody staining procedures (see reference 4 and Fig. 5). All the individual measles virus components exhibited consistently similar behavior in reaction to sera from several patients

with SSPE. SSPE sera specifically immunoprecipitated the 70,000 (P)-, 60,000 (N)-, and 35,000 (M)-molecular-weight polypeptides in acutely infected cells. Notably, however, the 78,000-molecular weight (G) viral component did not react with SSPE serum (Fig. 1 and 2).

That the 78,000-molecular-weight (G) protein is glycosylated (9, 17, 19) was confirmed by labeling infected and uninfected cells with [^3H]-glucosamine, followed by their extraction, immunoprecipitation, and electrophoresis on polyacrylamide gels. The polypeptide appears as the major new glycosylated component after infection (Fig. 2). The 78,000-molecular-weight (G) polypeptide was the sole glycosylated measles virus polypeptide observed on immunoprecipitation of [^3H]-glucosamine-labeled extracts with guinea pig antimeasles serum. As seen with [^{35}S]-methionine-labeled immunoprecipitates, sera from three individual SSPE patients did not precipitate the [^3H]-glucosamine-labeled 78,000-molecular-weight (G) moiety.

(ii) **Cell-free translation of mRNA.** Polyadenylic acid-containing RNA isolated from infected and uninfected cells has been used, as a first step, to direct polypeptide synthesis in the micrococcal nuclease-treated reticulocyte lysate system (12). Autoradiograms of polypeptides synthesized in measles virus-infected and uninfected cellular mRNA are presented in Fig. 3. The products of the infected cellular mRNA contain several polypeptides having molecular weights of 70,000, 60,000, 40,000, and 35,000, which are not found in the uninfected cellular mRNA. These weights correspond to those of some of the viral polypeptides found in infected cell extracts. The 60,000- and 70,000-molecular-weight products comigrated electrophoretically with the 60,000 (N)- and 70,000 (P)-molecular-weight viral proteins in purified nucleocapsids (Fig. 3).

The 78,000-molecular-weight glycosylated polypeptide was not detected among the *in vitro* products of infected cellular mRNA. Thus, the non-glycosylated polypeptide precursor of the 78,000-molecular-weight protein may have a mobility similar to that of one of the other viral products.

Partial chymotryptic digestion (3) of the 60,000-molecular-weight (N) purified protein yielded 10 detectable peptides when analyzed by polyacrylamide gel electrophoresis. After the same treatment of the 60,000-molecular-weight cell-free product of infected CV-1 cellular mRNA, a similar proteolysis pattern was observed (Fig. 4); 9 of the 10 fragments comigrated electrophoretically. Thus, the 60,000-molecular-weight (N) measles polypeptide synthesized in intact cells is closely related to the 60,000-mo-

FIG. 1. Measles virus polypeptide synthesis in lytically infected cells. Measles virus-infected and uninfected CV-1 cells were labeled with [^{35}S]-methionine (10 $\mu\text{Ci}/\text{ml}$) 24 h postinfection for 1 h. Cells were washed with reticulocyte standard buffer, extracted with reticulocyte standard buffer containing 0.5% Nonidet P-40, and either directly treated with electrophoresis sample buffer or immunoprecipitated as described in the text. (a) Infected cell extract; (b) uninfected cell extract; (c) guinea pig antimeasles virus immunoprecipitate of (a); (d) guinea pig anti-measles virus immunoprecipitate of (b); (e) SSPE serum immunoprecipitate of (a); (f) SSPE serum immunoprecipitate of (b).

FIG. 2. Immunoprecipitation of [^3H]glucosamine-labeled proteins from measles virus-infected cells. CV-1 cells were labeled with [^3H]glucosamine from 24 to 30 h postinfection, followed by Nonidet P-40 extraction and immunoprecipitation as described in the text. Immunoprecipitates were subjected to SDS-polyacrylamide gel electrophoresis followed by fluorography of the dried gel. (a) [^3H]glucosamine-labeled infected cell extract; (b) [^3H]glucosamine-labeled uninfected cell extract; (c) guinea pig antimeasles immunoprecipitate of [^3H]glucosamine-labeled infected cell extract; (d) SSPE serum immunoprecipitate of [^3H]glucosamine-labeled infected cell extract.

lecular-weight cell-free product synthesized in vitro in response to mRNA from measles virus-infected cells.

Measles virus proteins in persistently infected HeLa cells. (i) Synthesis. Two persistently infected HeLa cell lines, CI-11 and CI-11A, were tested for measles virus antigens by the indirect fluorescent staining technique, using human antimeasles and fluorescein isothiocyanate-conjugated rabbit anti-human gamma globulin. Under our conditions, 95% of the cell stained positively (Fig. 5). In addition, it was possible to purify nucleocapsid complexes from HeLa lines CI-11 and CI-11A, but in lesser amounts than were obtained from lytically infected monkey CV-1 cells (data not shown). When extracts of

[^{35}S]methionine-labeled persistent lines were subjected to SDS-polyacrylamide gel electrophoresis, no marked difference was found in the pattern of polypeptides synthesized in uninfected HeLa cells or in line CI-11 or CI-11A. However, guinea pig antimeasles virus serum immunoprecipitated three major polypeptides from CI-11 and CI-11A but not from uninfected HeLa extracts. The molecular weights of these polypeptides were 78,000, 70,000, and 60,000, and they comigrated electrophoretically with measles virus proteins similarly isolated from lytically infected CV-1 cells (Fig. 6). Partial proteolysis of the 60,000-molecular-weight form indicated that it contains sequences common to the 60,000-molecular-weight polypeptide synthe-

FIG. 3. *In vitro* synthesis of measles virus polypeptides. [^{35}S]methionine-labeled products were synthesized (a) in the absence of added RNA, (b) by mRNA direction from uninfected cells (2 μg of RNA per 25- μl reaction volume), or (c) by mRNA direction from measles virus-infected cells (2 μg of RNA per 25- μl reaction volume). Present in the same slab gel was (d) purified measles nucleocapsid labeled *in vivo* with [^{35}S]methionine. Gels were stained with Coomassie brilliant blue, dried, and autoradiographed for 1 day. Molecular weights (MW) as shown were calculated using standards of known molecular weights on the same slab gel. Markers used were phosphorylase (94,000 MW), bovine serum albumin (68,000 MW), beef liver catalase (60,000 MW), and gamma globulin, heavy chain (50,000 MW).

FIG. 4. Partial proteolysis of the 60,000-molecular-weight measles (M) protein. Authentic nucleoprotein purified from monkey cells (CV-1) acutely infected with measles virus and chronically infected HeLa cells (CI-11A) were obtained from SDS-polyacrylamide gels as described in the text. The 60,000-molecular-weight protein, synthesized in a cell-free system, was isolated by immunoprecipitation of [35 S]methionine polypeptides with guinea pig antimeasles serum, followed by SDS-polyacrylamide gel electrophoresis. The 60,000-molecular-weight polypeptide in each case was excised, incubated with 2 μ g of α -chymotrypsin for 30 min in SDS-gel buffer, and applied onto a 15% SDS-polyacrylamide gel. Autoradiograms (5 days) show: (A) *in vivo* (acutely infected) 60,000-molecular-weight nucleoprotein incubated (1) without enzyme and (2) with α -chymotrypsin; (B) *in vitro* product incubated (1) without enzyme and (2) with enzyme; (C) *in vivo* (chronically infected) product (1) without enzyme and (2) with α -chymotrypsin.

sized *in vivo* or *in vitro* from lytically infected cells (Fig. 4). In addition, a polypeptide which migrated somewhat slower than the 35,000-molecular-weight (M) protein of infected CV-1 cells was also immunoprecipitated. Similar experiments with [3 H]glucosamine demonstrated that the 78,000-molecular-weight viral protein immunoprecipitated from CI-11 and CI-11A is also glycosylated (S. Rozenblatt and O. Pinhasi, manuscript in preparation).

(ii) **Cell-free translation of mRNA.** Polyadenylic acid-containing mRNA was isolated from persistently infected cells and translated in the reticulocyte cell-free system. When products directed by mRNA from measles virus-infected CV-1 cells or the persistent HeLa cell lines CI-11 and CI-11A were analyzed by polyacrylamide gel electrophoresis (either directly or after immunoprecipitation with either guinea pig antimeasles serum or SSPE serum), it was found that the 70,000-, 60,000-, and 35,000-molecular-weight viral polypeptides were far more promi-

nent among the total products directed by mRNA from infected CV-1 cellular mRNA than by mRNA from the two persistent HeLa cell lines. Guinea pig antimeasles virus serum immunoprecipitates these three polypeptides from the products of the mRNA's from either acutely or chronically infected cells.

When the same series of cell-free products was reacted with SSPE serum, while the 60,000-molecular-weight polypeptide was immunoprecipitated to the same extent as with guinea pig antimeasles virus serum, the immunoprecipitation of the 70,000-molecular-weight polypeptide was comparatively greatly reduced (Fig. 7). Surprisingly, the 35,000-molecular-weight protein did not react at all with SSPE serum. These results were obtained repeatedly with sera from several patients with SSPE. The differences in immunoreactivity between guinea pig antimeasles virus serum and SSPE serum were observed with the cell-free products of mRNA from either lytically or persistently infected cells. It was also found that SSPE serum does not react with the M protein from extracts of persistently infected cells (data not shown).

Table 1 summarizes the information obtained in the experiments reported here on the immunoreactivity of the 78,000 (G)-, 70,000 (P)-, 60,000 (N)-, and 35,000 (M)-molecular-weight measles polypeptides. It should be noted that the G protein in the form of a 78,000-molecular-weight polypeptide was never detected among the *in vitro* translation products.

DISCUSSION

We have attempted to assess the involvement of measles virus in SSPE. SSPE serum specifically immunoprecipitated the measles virus RNA cell-free products, whereas sera from humans with no record of measles did not. Our finding that viral proteins extracted from acutely infected measles virus cells are sometimes precipitated with serum from nonimmunized guinea pigs may indicate a form of nonspecific trapping of the nucleocapsid or other viral complexes. However, products synthesized *in vitro* were specifically immunoprecipitated with guinea pig antimeasles virus serum but never with the control sera. Thus, the *in vitro* translation system has a unique advantage for assessing immunoreactivity of measles virus proteins.

Our results confirm that the 78,000-molecular-weight protein is glycosylated (9, 17, 19), and this property may be related to its altered immunoreactivity. Although under all the conditions tested, the 60,000-molecular-weight (N) and 70,000-molecular-weight (P) polypeptides were precipitated, SSPE serum did not react

FIG. 5. Intracellular viral immunofluorescence of HeLa cells. The presence of measles virus-specific antigens in acutely and chronically infected cells was determined by the indirect immunofluorescence technique with SSPE serum and fluorescein isothiocyanate-conjugated rabbit anti-human gamma globulin. (A) Mock-infected cells; (B) lytically infected CV-1 line; (C) persistently infected Cl-11 line; (D) persistently infected Cl-11A line.

with the 78,000-molecular-weight (G) or, except in acutely infected cells, the 35,000-molecular-weight (M) polypeptides. The glycosyl moiety may be the major antigenic determinant for the 78,000-molecular-weight polypeptide.

The virus produced in brain tissue of SSPE patients may contain a different glycosyl moiety which elicits antibodies that are not cross-reactive with the 78,000-molecular-weight component produced in acute infection. Alternatively, although infectious measles-like virus (SSPE virus) containing the 78,000-molecular-weight (G) protein can be rescued from brain tissue of SSPE patients by cocultivation (7, 11), the protein may not be synthesized in this tissue. Mature particles are not released from the cells of SSPE patients and budding particles have not been detected in electron micrographs, although nucleocapsid complexes have been observed (16).

The glycosylation of the 78,000-molecular-weight (G) polypeptide may also explain why this protein is not detected among the cell-free products of mRNA from either acutely or persistently infected cells. Since glycosylation is known in other cases to retard the electrophoretic

mobility of a polypeptide (5), and since it is unlikely that the reticulocyte translation system can glycosylate a polypeptide *in vitro*, the non-glycosylated form of the 78,000-molecular-weight (G) protein probably has a different electrophoretic mobility. The data of Fig. 7 indicate that the nonglycosylated form comigrates electrophoretically with the 70,000-molecular-weight (P) protein when the cell-free products are immunoprecipitated with SSPE serum. Considerably less 70,000-molecular-weight product was immunoprecipitated with SSPE serum than with guinea pig antimeasles serum. This would be understandable if the non-glycosylated form of the 78,000-molecular-weight protein has an electrophoretic mobility similar to that of the 70,000-molecular-weight protein and if the polypeptide portion of the G protein is weakly, or not at all, immunoreactive with SSPE serum.

Differences in the electrophoretic mobility and immunospecificity of the 35,000-molecular-weight (M) protein noted in our experiments are particularly interesting in light of recent reports of an altered M protein in SSPE virus isolated by cocultivation of brain tissue of SSPE patients

FIG. 6. Measles virus polypeptide synthesis in persistently infected cells. Persistently infected and uninfected HeLa cells were labeled with [35 S]methionine (20 μ Ci/ml) for 1 h. Cells were washed with reticulocyte standard buffer, and proteins were extracted as described in the legend to Fig. 1. (a) Uninfected cell extract; (b) persistently infected CI-11 cell extract; (c) persistently infected CI-11A cell extract; (d) guinea pig antimeasles virus immunoprecipitate of (a); (e) guinea pig antimeasles virus immunoprecipitate of (b); (f) guinea pig antimeasles virus immunoprecipitate of (c); (g) guinea pig antimeasles virus immunoprecipitate of acutely infected cell extract.

FIG. 7. Measles virus polypeptides synthesized *in vitro* in response to mRNA from acutely and chronically infected cells. (a) [35 S]methionine-labeled products directed by mRNA from measles virus acutely infected cells (2 μ g of RNA per 25- μ l reaction volume); (b) guinea pig antimeasles immunoprecipitate of (a); (c) SSPE serum immunoprecipitate of (a); (d) [35 S]methionine-labeled products directed by mRNA from chronically infected CI-11 cells with measles virus (2 μ g of RNA per 25- μ l reaction volume); (e) guinea pig antimeasles virus immunoprecipitate of (d); (f) SSPE serum immunoprecipitate of (d); (g) [35 S]methionine-labeled products directed by mRNA from CI-11A cells chronically infected with measles virus (2 μ g of RNA per 25- μ l reaction volume); (h) guinea pig antimeasles virus immunoprecipitate of (g); (i) SSPE serum immunoprecipitate of (g).

TABLE 1. Immunoreactivity of measles virus polypeptides synthesized *in vivo* or *in vitro* from acutely or persistently infected cells

Measles virus poly-peptide (mol wt. $\times 10^{-3}$)	Guinea pig antimeasles				SSPE serum			
	Acute		Chronic		Acute		Chronic	
	In vivo	In vitro	In vivo	In vitro	In vivo	In vitro	In vivo	In vitro
78 (G)	+	—	+	—	—	—	—	—
70 (P)	+	+	+	+	+	+	+	+
60 (N)	+	+	+	+	+	+	+	+
35 (M)	+	+	+	+	+	—	—	—

having a lower electrophoretic mobility than the M protein from measles virus (6, 18). Also, there is no cross-reactivity between purified M protein from measles and SSPE virus isolates (6). In seemingly analogous fashion, the putative M protein isolated from the persistent lines had a reduced electrophoretic mobility when compared to the M protein in acute infection. Furthermore, SSPE serum did not react with the M protein synthesized *in vitro* or in persistently infected cells. Although SSPE serum did react with the M protein in acutely infected cells, this could well be an example of the trapping of the viral complex in the immunoprecipitate described above.

These observations of differences in immunoreactivity of viral polypeptides synthesized *in vivo* and *in vitro* encourage the continuation of our program to investigate the structural analysis of the proteolytic products of these different viral components in different biological states.

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