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Poliovirus Infection Results in Structural Alteration of a Microtubule-Associated Protein

MICHELLE JOACHIMS AND DIANE ETCHISON*

Department of Microbiology, Molecular Genetics and Immunology, University of Kansas Medical Center, Kansas City, Kansas 66160

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Poliovirus infection results in profound changes in cellular metabolism and architecture. To identify alterations in cellular proteins following poliovirus infection which might account for these changes, monoclonal antibodies were prepared by screening for differences in antigen pattern in infected and uninfected cell lysates. Further characterization of the antigen of one such antibody (25 C C1) is described in this report. The 25 C C1 antigen is a cytoskeleton-associated protein which decreases in size 4 to 5 h postinfection. It copurifies with some of the protein synthesis initiation factors but not with eucaryotic initiation factor (eIF)-4F, the p220 subunit of which is cleaved following infection (D. Etchison, S. C. Milburn, I. Edery, N. Sonenberg, and J. W. B. Hershey, J. Biol. Chem. 257:14806–14810, 1982). Unlike alteration of p220, alteration of the 25 C C1 antigen is not due to a protease which can be detected by cell lysate mixing experiments. Alteration of the antigen occurs during purification, suggesting progressive proteolysis, but the alteration is more extensive in preparations from infected cells than in those from uninfected cells. A recombinant phage expressing the antigenic determinant was isolated from a human fibroblast cDNA library, and the sequence of the cDNA insert was found to be entirely contained within the established sequence of microtubule-associated protein (MAP) 4 (R. R. West, K. M. Tenbarge, and J. B. Olmsted, J. Biol. Chem. 266:21886–21896, 1991). The antigen distribution, as detected by indirect immunofluorescence, was similar to, but more diffuse than, the distribution of tubulin. The antibody recognized the largest abundant HeLa cell MAP, which copurified with tubulin after three cycles of polymerization-depolymerization, thus confirming the identity of the antigen as MAP 4. These results indicate that poliovirus infection of HeLa cells affects the structural integrity of a cytoskeletal protein, MAP 4.

Host cell alterations following poliovirus infection have been the subject of numerous studies, but to date only a few of these changes have been correlated with specific alterations of host cell proteins. Some of the virus-induced alterations which have been identified include the selective shutoff of host protein and RNA synthesis (32), an increased synthesis of phospholipids (43), and changes in cell membrane permeability (9), as well as profound changes in host cell architecture (14). The mechanism of inhibition of host mRNA translation in infected cells has been correlated with the indirect cleavage of a cellular protein, p220, by the viral protease 2A (19, 31). Shutoff of host cell transcription has been correlated with dephosphorylation or limited degradation of host RNA polymerases and transcription factors (11, 12, 15, 30). However, little is known about the specific alterations which lead to changes in cell membrane permeability or cell architecture.

Alterations in the cytoskeleton and cellular membranes following poliovirus infection result in a rearrangement of cellular architecture which is broadly termed the cytopathic effect. Intermediate filaments are remodeled (33), the nucleus becomes crenated, and there is an accumulation of smooth membranous structures in the cytoplasm (8, 38). Poliovirus replication complexes are associated with these membranous structures (3), which appear in response to the presence of the viral protein 2BC (2). An inhibitor of Golgi membrane translocation, brefeldin A, has been shown to drastically inhibit poliovirus replication, suggesting that membrane translocation plays a role in viral RNA synthesis (37). Less is known about the possible role of cytoskeletal changes in viral growth and cytopathology.

In an attempt to elucidate the changes in cellular proteins involved in the shutoff of host protein synthesis, monoclonal antibodies were prepared by immunizing mice with a partially purified initiation factor preparation and hybridomas were selected by screening for differences in immunoblot reactivity to uninfected and poliovirus-infected cell lysates. One hybridoma produced antibodies capable of recognizing the p220 subunit of eucaryotic initiation factor (eIF)-4F, which is cleaved in infected cells (17). Its subcellular distribution as detected by indirect immunofluorescence was primarily cytoplasmic with a minor nuclear association, but no evidence for a specific association with the cytoskeleton was apparent. Lysate mixing experiments established the existence of an activity in infected cells which was capable of cleaving the intact antigen in uninfected lysates, and cleavage was demonstrated to occur early in the infectious cycle, with kinetics resembling those of host cell shutoff (19). The monoclonal antibody approach is therefore a useful method for identifying alterations induced by poliovirus infection, and it also provides a basic characterization of the antigen which can help to confirm the identity of the gene isolated during later cloning efforts.

In this report, we describe another monoclonal antibody, 25 C C1, which detected differences between infected cell lysates and uninfected cell lysates. This antibody was prepared during the same fusion procedure as the one which produced the anti-p220 antibody (17). In this study, the antigen recognized by the second antibody was further characterized as a cytoskeletal protein that is structurally altered after poliovirus infection. Its size, subcellular distri-

* Corresponding author.

bution, and immunofluorescence pattern helped to confirm the conclusions of genetic cloning, which have revealed its likely identity to be microtubule-associated protein (MAP) 4. The possible involvement of an alteration in MAP 4 with poliovirus cytopathology is discussed below.

MATERIALS AND METHODS

Cells and virus. HeLa cells were grown as suspension culture (HeLa S3) in Joklik's modified minimal essential medium supplemented with 6% calf serum and 2 mM glutamine or as monolayer (HeLa CG) culture in regular minimal essential medium supplemented with calf serum and glutamine. Poliovirus type 1 (Mahoney strain) was grown and purified as described previously (16). For infection of suspension cultures, cells were concentrated 10-fold in medium lacking serum and infected at a multiplicity of 100 PFU per cell. The cells were incubated at 37°C for 30 min, with stirring, and prewarmed serum was then added to a final concentration of 5%. For infection of monolayer cells, the medium was removed, fresh medium containing virus was added, and the plate was rocked every 10 min for 30 min. Additional medium containing serum was then added to give a final concentration of 5%.

Monoclonal antibodies and immunoblotting. Preparation of anti-p220 monoclonal antibody (25 A C2) has been described previously (17). The 25 C C1 cell line was cloned from hybridomas produced from the spleen cells from a mouse immunized with a preparation containing p220 (18). This fusion procedure also produced the anti-p220 hybridoma, 25 A C2. The antigen had been purified from the 0 to 40% ammonium sulfate fraction of a ribosomal salt wash of uninfected HeLa cells through a sucrose gradient fractionation procedure followed by DEAE chromatography. Hybridomas were selected by enzyme-linked immunosorbent assay (ELISA) with the immunizing antigen as the screening antigen and by immunoblot analysis of uninfected and poliovirus-infected postmitochondrial supernatants. The 25 C C1 antibody is an immunoglobulin M type and is used either as a mouse ascites preparation at a 1:5,000 dilution or was ammonium sulfate precipitated from growth medium and used at a 1:2,000 dilution. For immunofluorescence, the antibody was purified by ammonium sulfate precipitation and DEAE chromatography from a mouse ascites fluid.

Mouse monoclonal antibodies to sea urchin tubulin were prepared from a hybridoma cell line isolated by B. Neighbors (44) and purified by R. H. Himes (University of Kansas). Fluorescein-conjugated rabbit anti-mouse antibodies were purchased from Antibodies, Inc. (Davis, Calif.).

Subcellular fractionation. Subcellular fractions of HeLa S3 cells for immunoblot analysis were prepared according to standard procedures for purification of initiation factors, essentially as described by Etchison and Milburn (18). Briefly, frozen cells were thawed and homogenized in a Tissuemizer. After nuclei and mitochondria were removed by centrifugation, the supernatant (S10) was subjected to centrifugation at 100,000 × *g* for 2.5 h. The postribosomal supernatant (S100) was saved separately, and the ribosomal pellet was resuspended in ribosome buffer, incubated for 15 min in 500 mM KCl, and recentrifuged. The supernatant (ribosomal salt wash [RSW]) was fractionated by ammonium sulfate precipitation into an RSW A fraction (0 to 40%), which contains eIF-3, eIF-4F, and eIF-4B, and an RSW B fraction (40 to 70%).

To examine the possible cofractionation of 25 C C1 antigen with cytoskeleton, HeLa S3 cells were fractionated according to the general procedure of Fey et al. (21). Cells were harvested, washed with serum-free medium, and resuspended in 2 cell pellet volumes of cytoskeleton buffer (300 mM sucrose, 10 mM Tris [pH 6.8], 3 mM $MgCl_2$, 0.5% Triton X-100, 100 mM NaCl) for 10 min at 0°C. After centrifugation for 15 min at 10,000 rpm, the detergent-soluble supernatant (SOL) was saved and the pellet was resuspended in 2 cell pellet volumes of extraction buffer (250 mM ammonium sulfate, 300 mM sucrose, 10 mM Tris [pH 6.8], 0.5% Triton X-100). After recentrifugation, the cytoskeleton-associated supernatant (CSK) was saved and the nuclear pellet was resuspended in 1 cell pellet volume of nuclease buffer (50 mM NaCl, 300 mM sucrose, 10 mM Tris [pH 6.8], 3 mM $MgCl_2$, 0.5% Triton X-100). DNase I and RNase A were added to the resuspended nuclei to final concentrations of 100 μg/ml each, and after incubation for 20 min at room temperature, ammonium sulfate was added to a final concentration of 0.25 M and the suspension was centrifuged as before. The chromatin-associated supernatant (CHRM) was saved, and the pellet was resuspended in 1% sodium dodecyl sulfate (SDS) and heated to boiling for 2 min to prepare a nuclear matrix (NM) fraction.

Indirect immunofluorescence. Indirect immunofluorescence was performed as described elsewhere (17). Briefly, monolayer HeLa cells were grown on circular glass coverslips, and cells were prepared by a quick washing with phosphate-buffered saline (PBS) and then immersion for 5 min in acetone which had been prechilled to −90°C. After air drying, the coverslips were incubated with a 1/500 dilution of purified 25 C C1 antibodies or a 1:3,000 dilution of antitubulin antibodies and then with fluorescein isothiocyanate-conjugated goat anti-mouse antibodies, and the slides were mounted on a drop of *n*-propyl gallate in 70% glycerol–PBS. Slides were viewed by epi-illumination and photographed with Kodak Ektachrome ASA 400 slide film, and black-and-white photographs were reproduced from the color slides.

Genetic cloning. The 25 C C1 monoclonal antibody was used as an immunoscreening reagent to probe an oligo(dT)-primed human fibroblast (WI-38 cell) cDNA library in Uni-Zap (Stratagene) according to the manufacturer's directions. Five positive recombinant phage were identified, and each was expanded and rescreened three times. They all contained overlapping sequences, as detected by Southern analysis using the clone with the longest cDNA insert as a probe. The inserts were partially sequenced, and sequences were compared with gene sequences in the EMBL Gene Bank data base. One insert was 100% identical to nucleotides 664 to 1210 of the sequence for human MAP 4 (accession no. M64571).

Tubulin purification. Tubulin from HeLa S3 cells was isolated essentially as described by Bulinski (5). Briefly, cells were harvested and washed twice in PBS and once in PDEMG buffer [0.1 M piperazine-*N*,*N*′-bis(2-ethanesulfonic acid) (PIPES) (pH 6.94), 1 mM dithiothreitol, 1 mM ethylene glycol-bis(β-aminoethyl ether)-*N*,*N*,*N*′,*N*′-tetraacetic acid (EGTA), 1 mM $MgSO_4$, and 0.1 mM GTP]. Cells were resuspended in an equal volume of PDEMG buffer and sonicated with three 30-s bursts. The cell lysate was then centrifuged at 48,000 × *g* for 30 min at 4°C, and the cleared lysate was used for two to three cycles of polymerization-depolymerization. A cycle of reversible polymerization was initiated by adding 1 mM GTP to the lysate and incubating the mixture for 30 min at 37°C. The polymerized microtubules were collected by centrifugation at 48,000 × *g* for 30 min at 37°C. The pellet was resuspended in 1/2 to 1/5 volume of PDEMG buffer and chilled on ice for 30 min to depoly-

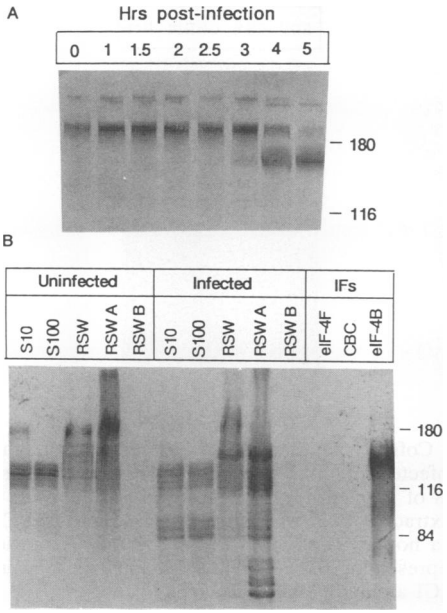

FIG. 1. Postinfection alteration time course and subcellular distribution of the 25 C C1 antigen. (A) HeLa cells in monolayer culture were infected with poliovirus as described in Materials and Methods, and one dish of infected cells was harvested at each of the indicated times after infection by lysing the washed monolayer with gel sample buffer. Cell lysates were gathered by scraping, and the samples were heated to boiling for 3 min and loaded onto 8% polyacrylamide–SDS gels. The gel was electrophoresed, and proteins were electroblotted onto nitrocellulose. Immunoblots were processed with 25 C C1 monoclonal antibodies. Immunoblotting procedures have been described previously (20). (B) Uninfected and 4-h-postinfection HeLa S3 cells were harvested and processed for initiation factor preparation by standard procedures (18). A portion of each cell fraction containing 0.3 A_{280} units was analyzed by immunoblot with 25 C C1 antibody. Purified initiation factors (IFs) were prepared as follows: eIF-4F was purified by cap-affinity chromatography as previously described (20); the eIF-4B purification procedure was that of Benne et al. (1); and cap-binding complex (CBC) is a cytosolic cap-affinity-purified preparation from the postribosomal supernatant of uninfected cells. Numbers on the right of the figure are size markers (kilodaltons).

merize microtubules. The suspension was then clarified by centrifugation and subjected to two additional rounds of polymerization-depolymerization.

RESULTS

In order to identify cellular proteins that are altered after poliovirus infection, hybridomas were prepared by using mice that had been immunized with a partially purified preparation containing the p220 subunit of eIF-4F (17). Several hybridomas which produced antibodies directed against antigens that were altered in infected cells were identified. The antigen recognized by one of these, 25 C C1, was identified by immunoblot of the lysates of monolayer cells harvested at different times after infection. Figure 1A shows that the 25 C C1 antigen remained relatively intact until 4 to 5 h after infection and that the primary form of the antigen in uninfected cells was a polypeptide of approximately 210 kDa, with an additional minor form of approximately 300 kDa. By 3 h postinfection, which would correspond with the peak of viral RNA and protein synthesis and the appearance of membranous structures and cytoskeletal changes, the altered form of the 25 C C1 antigen first appeared. By 4 h postinfection, approximately 50% of the antigen was altered, and by 5 h virtually none of the intact 210-kDa antigen was found. The larger minor form of the antigen was also altered, with similar kinetics, producing a slightly smaller form of similar abundance. These results demonstrate that the alteration of the 25 C C1 antigen occurs relatively late after infection and, therefore, does not correlate with early-onset changes such as p220 cleavage or shutoff of host cell protein synthesis.

Subcellular distribution. To examine the subcellular distribution of the antigen in uninfected HeLa suspension cells and to determine whether there is a change in distribution in infected cells, HeLa S3 cells were fractionated by standard procedures for initiation factor purification into a postmitochondrial supernatant (S10), a postribosomal supernatant (S100), a high-salt wash of the ribosomal pellet (RSW), and two ammonium sulfate-precipitated fractions of the RSW (RSW A and B). The presence of the 25 C C1 antigen in these fractions was determined by immunoblot (Fig. 1B). In addition, the presence of the antigen was examined in cap-affinity-purified preparations of eIF-4F and a cytosolic cap-binding complex purified from the postribosomal supernatant of uninfected cells, as well as in an eIF-4B preparation. In the S10 prepared from suspension cells, the primary antigen was approximately 150 kDa in size, although a minor amount appeared at 180 kDa. The difference between the sizes of the antigen from monolayer cells and the antigen from suspension cells may be due to the increased handling of the suspension cell fractions (see Discussion). The suspension cells were prepared by mechanical disruption with a Tissuemizer, whereas the monolayer cells had been lysed in situ with SDS. The larger form of the 25 C C1 antigen copurified with ribosomes and was the major form of the antigen in the RSW. In infected cells harvested 4 h after infection, the immunoblot pattern included a smaller set of polypeptides, the most abundant of which was approximately 80 to 90 kDa in size, and this form of the antigen was not found in uninfected cell preparations. Again, the larger forms copurified with the RSW, while an even smaller set of polypeptides (55 to 65 kDa) appeared after ammonium sulfate fractionation. In spite of the fact that the immunizing antigen had copurified with p220, no 25 C C1 antigen was found in cap-affinity chromatography preparations, one of which contains p220 (eIF-4F). Instead, the antigen was found in at least one eIF-4B preparation. However, not all eIF-4B preparations contained the antigen (data not shown). These data also indicate that the structural alteration in this antigen which occurs in infected cells does not change its subcellular distribution.

The 25 C C1 antigen alteration is virus specific. Since the p220 subunit of eIF-4F could be cleaved in vitro by incubation of infected lysates containing the p220 protease with uninfected cell lysates containing intact p220, a similar analysis was performed to examine the nature of the alteration of the 25 C C1 antigen. Incubation of 4-h-postinfection lysates of suspension cells with uninfected lysates containing the unaltered form of the antigen had no effect on the gel mobility of the antigen (Fig. 2). There was no decrease in the 180- and 150-kDa antigens that were provided by the uninfected cells upon incubation for 60 min with infected lysate, and there was no increase in the smaller antigenic forms characteristic of infected lysates after incubation. Similar results were obtained with 5-h-postinfection lysates of

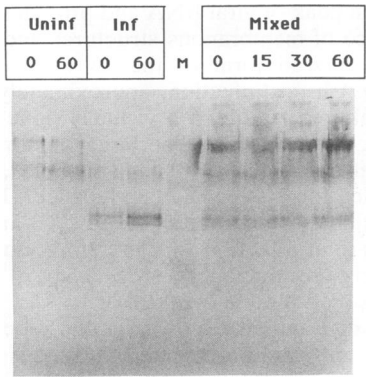

FIG. 2. 25 C C1 antigen integrity is unaffected by incubation with infected cell lysates. S10 cell extracts were prepared according to standard initiation factor preparation procedures from uninfected (Uninf) or 4-h-postinfection (Inf) HeLa S3 cells. For this experiment, buffers did not contain phenylmethylsulfonyl fluoride. Portions of each lysate containing 0.2 A_{280} units were incubated either separately or together (Mixed) for the indicated times (numbers at top, in minutes) at 37°C, and the incubation was terminated by adding 1/3 volume of 4× gel sample buffer and boiling. Samples were then applied to 10% polyacrylamide–SDS gels and further processed for immunoblotting with the 25 C C1 antigen as described in the legend to Fig. 1. Lane M is prestained markers consisting of polypeptides of the following M_rs: 210,000, 116,000, 84,000, 58,000, and 48,000 (Sigma Chemical Co., St. Louis, Mo.).

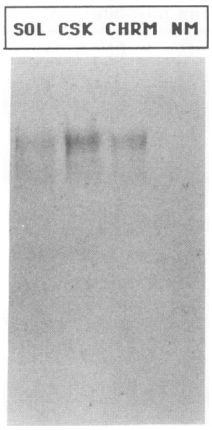

FIG. 3. Cofractionation of the 25 C C1 antigen with the cytoskeleton. Uninfected HeLa S3 cells were fractionated according to the procedures of Fey et al. (21) into a SOL cytoplasmic fraction, a high-salt-extracted CSK fraction, a nuclease-released CHRM fraction, and a hot SDS-solubilized NM fraction. A portion of each fraction representing 0.1 A_{280} units was analyzed by immunoblot with 25 C C1 antibody, as for previous figures.

monolayer cells (data not shown). Thus, in spite of the fact that the alteration of the 25 C C1 antigen resembles proteolysis, it is not accomplished by a protease which survives cell fractionation. These results also indicate that the agent which alters 25 C C1 is completely distinct from the protease which cleaves p220. Furthermore, the alteration of the 25 C C1 antigen does not occur in infected cells treated with guanidine (data not shown). Guanidine inhibits viral RNA replication (25, 42) but does not prevent shutoff of host protein synthesis or p220 cleavage (4). It is likely, therefore, that the viral mediator of the 25 C C1 antigen alteration accumulates during infection and is not an enzymatic activity produced during the initial translation of input viral RNA.

Association of the 25 C C1 antigen with the cytoskeleton. The heterogeneous nature of the 25 C C1 antigen suggested that it might be a cytoskeletal protein. To examine this possibility, cells were extracted according to the procedure of Fey et al. (21) to provide fractions containing the SOL, CSK, CHRM, and NM proteins (Fig. 3). When samples of these fractions were analyzed for the 25 C C1 antigen by immunoblot, the SOL, CSK, and CHRM proteins contained detectable antigen but the NM protein did not. The presence of antigen in the CSK fraction is consistent with its association with the cytoskeleton, and its presence in the SOL fraction as well suggested that there is a significant population of soluble forms of the antigen. This distribution is similar to that of actin, a portion of which is always in a depolymerized soluble form (22). The 25 C C1 antigen was also found in the CHRM fraction, indicating that a portion of this antigen is located in the nucleus. Since there was no trace of the antigen in the nuclear matrix, however, it appears that the nuclear form of the antigen is not tightly bound to the nuclear matrix. These results indicate that the 25 C C1 antigen is at least partially physically associated with cytoskeletal structures.

Sedimentation analysis of the 25 C C1 antigen suggests that it is associated with polymerized structures. To examine the possible copurification of the 25 C C1 antigen with eIF-3 or with the poliovirus-altered form of eIF-4F from infected cells, an RSW A from 4-h-postinfection HeLa S3 cells was fractionated by sucrose gradient sedimentation under conditions which normally separate eIF-3 from cleaved p220 (17) (Fig. 4). Immunoblot and ELISA analysis of this gradient with p170–eIF-3 and p220 monoclonal antibodies placed the eIF-3 (17S) peak at fraction 9 and the cleaved p220 peak at fraction 22 (data not shown). The 25 C C1 antigen from infected cells sedimented throughout the gradient, but the smaller forms were consistently found near the top of the gradient. There was no evidence for a specific size distribution of the larger forms of the antigen, suggesting that they were associated with structures having a wide range of sedimentation values. This result is most consistent with an association of the antigen with polymeric structures. The smaller forms of the antigen are likely to be soluble forms

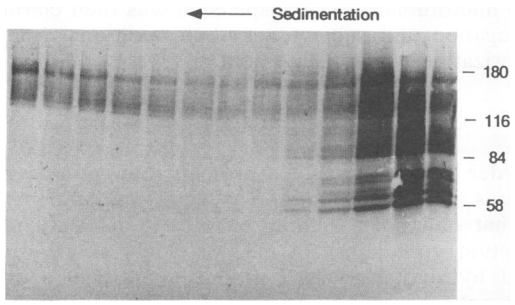

FIG. 4. Sedimentation analysis of the 25 C C1 antigen. RSW A was prepared from HeLa S3 cells 4 h after poliovirus infection, and 120 A_{280} units, from approximately 10^7 cells, were loaded onto a 10 to 30% sucrose gradient in buffer A-100. The gradient was centrifuged for 36 h at 25,000 rpm in a Beckman SW 28 rotor. It was then fractionated into 26 fractions, and 20 μl each of every other fraction (2 to 26) was analyzed by immunoblot with 25 C C1 antibodies as described in the legend to Fig. 1. Numbers on the right of the figure are size markers (kilodaltons).

rather than polymer associated. These data suggest that infection does not abolish the association of the antigen with a heterogeneously sized polymer structure but that the smaller forms of the antigen do not remain associated.

Genetic cloning. We next used the 25 C C1 monoclonal antibody as a screening reagent in order to genetically clone the antigen. A fibroblast (WI-38 cell) cDNA library was probed by immunoscreening techniques, and five positive recombinants were identified. The details of this analysis will be described in a subsequent report. All five recombinants shared sequences, as detected by Southern analysis, and one of them was sequenced in full. The cDNA insert was found to be 100% identical to that of MAP 4 between nucleotides 664 and 1210 of the 5,037-nucleotide sequence reported by West et al. (52).

Cellular distribution of the 25 C C1 antigen as detected by indirect immunofluorescence. To confirm the identity of the 25 C C1 antigen as MAP 4, the monoclonal antibody was used to examine the cellular distribution of the antigen by indirect immunofluorescence, and the pattern obtained was compared to that of tubulin (Fig. 5). Since MAPs are constituents of microtubules, the cellular patterns of distribution of MAPs and tubulins are similar (7). Figure 5 shows the comparative immunofluorescence patterns of these two antigens. The tubulin pattern in uninfected HeLa cells consisted of typical fine filaments and showed a distinctive concentration of the antigen along the chromatids during mitosis (Fig. 5A). The 25 C C1 pattern was more diffuse, suggesting that only a portion of the antigen was copolymerized with tubulin (Fig. 5B). The diffuse pattern may be partly due to the fact that this antibody is an immunoglobulin M antibody and its immunofluorescence pattern might be expected to be less defined than that of an immunoglobulin G antibody. It may also be due to the existence of a large proportion of soluble MAP 4 in these cells, as suggested by the results of the preceding experiment. HeLa cells are a relatively poor source of microtubules. Some filaments were apparent, however, and there was a concentration of the antigen in dividing cells which resembled the distribution of tubulin. In addition, the 25 C C1 antigen was concentrated near the cell periphery as well as in the midbody structure which forms between cells undergoing the final stage of cell division, or cytokinesis (Fig. 5C). The midbody structure is known to contain both tubulin and MAP 4 (48, 49).

Copurification of the 25 C C1 antigen with microtubules. As a final confirmation of the identity of the 25 C C1 antigen with MAP 4, microtubules were purified by successive rounds of polymerization and depolymerization, and the purified material was analyzed for the presence of the 25 C C1 antigen by immunoblot. Figure 6A shows a polyacrylamide gel analysis of the different stages of microtubule purification which demonstrates the increasing relative abundance of tubulin with each round of polymerization. At least two MAPs can be identified in the final purified material by Coomassie blue staining. Immunoblot analysis of those same fractions showed that the 25 C C1 antigen also copurified with microtubules and that the majority of the antigen was identical in size to the largest and most abundant protein which copurified with tubulin (Fig. 6B). This method of microtubule purification results in the copurification of two prominant MAPs from HeLa cells, and MAP 4 is the larger (200 to 220 kDa) (6). These results indicate that the 25 C C1 antigen is identical to MAP 4.

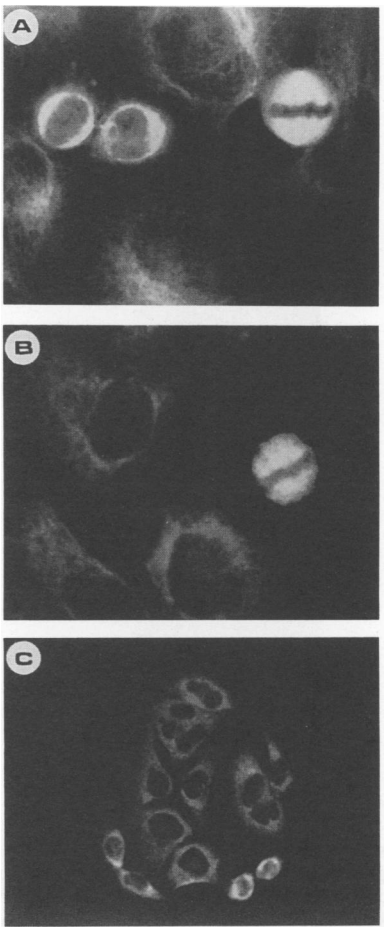

FIG. 5. Antigen distribution analyzed by indirect immunofluorescence. HeLa monolayer cells were grown on coverslips and processed for immunofluorescence according to procedures described in Materials and Methods. Since it was not possible to focus on both the cytoplasmic filaments and the chromatids in rounded-up dividing cells, we chose to focus on filaments. (A) Antitubulin antibodies; (B and C) 25 C C1 purified antibodies. Images were photographed with a Zeiss 100× objective (A and B) and a Zeiss 40× objective (C).

DISCUSSION

In this report, we describe the monoclonal antibody-mediated characterization of a CSK host cell protein that is altered during poliovirus infection. Genetic cloning identified the 25 C C1 antigen as MAP 4, a protein originally found to be a 210-kDa polypeptide which copurifies with microtubules from HeLa cells (6, 52). The identity of the antigen with MAP 4 was confirmed by its copurification with microtubules during successive polymerization-depolymerization cycles. MAPs are a family of proteins which share a tubulin-binding domain and are primarily involved in stimulating the polymerization of tubulin into microtubules (13). Different MAPs appear to differ in their specificity for microtubules from different sources (40, 41). Some of the MAPs are also associated with motor functions which move particles along microtubules in a unidirectional manner. Alterations in MAPs have been described, including calpain- and cathepsin D-mediated cleavage (27, 36) and phosphorylation by various kinases including the cell cycle-associated MAP kinase

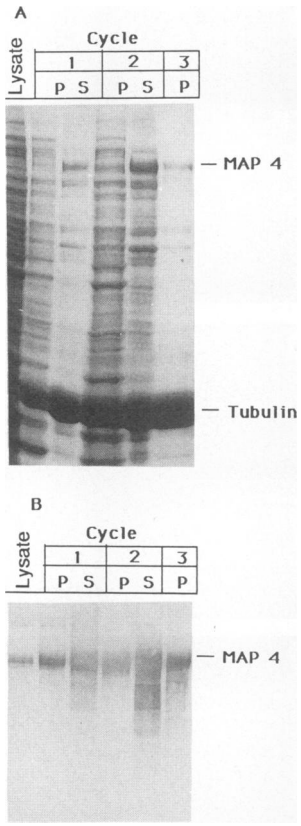

FIG. 6. Copurification of the 25 C C1 antigen with tubulin. Tubulin was purified from HeLa S3 cells, and immunoblots were analyzed by procedures described in Materials and Methods. Ten micrograms of each fraction of the resuspended polymerization step pellet (P) or the corresponding supernatant (S) of successive polymerization-depolymerization cycles was loaded onto 8% polyacrylamide–SDS gels. One gel was stained with Coomassie blue (A). The other was analyzed by immunoblot with the 25 C C1 antibody (B).

(45), but little is known about how these alterations affect cellular functions. Some of these functions are likely to include cell-cycle-stage-specific processes, and MAP 4 has been shown to undergo alterations in phosphorylation pattern in response to the onset of mitosis (48). The finding in this study that MAP 4 is structurally altered after poliovirus infection suggests the possibility that MAP 4 may also be involved in virus-specific processes.

At the present time, no microtubule-associated alterations in infected cells have been specifically identified. The only cytoskeletal changes that have been described appear to involve primarily intermediate filaments (33). However, membrane-associated changes have been studied in detail because of the role of cell membrane vesicles in viral replication, and cytoskeleton-membrane interactions could be involved in this process. Recently, an inhibitor of membrane translocation to the Golgi apparatus, brefeldin A, was found to drastically inhibit poliovirus replication (37). Since microtubules are involved in the organization of the Golgi complex and in the movement of membranous vesicles (47, 53), it is conceivable that alterations in MAPs might have an effect on vesicle movement.

In addition to their possible role in membrane-cytoskeleton interactions, MAPs have been shown to stimulate specific biochemical reactions, including DNA polymerase α activity (46) and vesicular stomatitis virus replication (24). These reports imply that MAPs might mediate nucleic acid-protein interactions, and since microtubules are essential for the formation of membrane-bound polysomes, MAPs may directly or indirectly be involved in formation of translational complexes (39, 49a).

Partial copurification of MAP 4 with the p220 subunit of eIF-4F during a standard initiation factor preparation, but not by cap-affinity chromatography of eIF-4F, suggests the possibility that the two proteins might be transiently associated with each other or that they might both be associated with common cellular structures. It has long been known that translating mRNAs are associated with the cytoskeleton and that inhibition of translation by poliovirus, or by cytochalasin, results in the release of host cell mRNA from the cytoskeleton (10, 28, 34, 39, 54). Furthermore, protein synthesis initiation factors are enriched in the cytoskeleton fraction which remains after extraction of cells with detergents (26). It is therefore intriguing to consider that MAP 4 might be a candidate mediator of translational apparatus-cytoskeleton interactions. In support of this hypothesis, we demonstrated that the MAP 4 antigen is found in preparations containing the RNA-binding initiation factor eIF-4B (Fig. 1B), and preliminary results suggest that the MAP 4 antigen affects RNA-binding activity associated with eIF-4B preparations (13a).

In spite of its similarity in size to the p220 subunit of eIF-4F, MAP 4 is distinct by several criteria. First, its structural alteration occurs later than that of p220 (19), occurring 4 to 5 h rather than 2 to 3 h postinfection. Second, its alteration cannot be accomplished in vitro by coincubation of uninfected and infected cell lysates; thus, the agent which alters MAP 4 antigen is not the same as that which cleaves p220. The MAP 4 antigen is not found in cap-binding complexes which contain p220, but it does copurify with eIF-4B. Furthermore, MAP 4 appears to be more fragile than p220, as indicated by its apparent degradation during purification procedures which have little effect on the integrity of p220.

The nature of the alteration in MAP 4 which occurs following poliovirus infection is not known. The apparent fragility of MAP 4 in uninfected cells suggests susceptibility to endogenous proteases (51). MAPs are known to be particularly sensitive to degradation in vitro under conditions in which tubulin remains intact (29). The increased fragility in poliovirus-infected cells might be due to increased activity of cellular proteases which are not sufficiently active to be detected in a cell lysate in the mixing experiments (Fig. 2). Alternatively, infection might alter the phosphorylation state of MAP 4 and increase its susceptibility to endogenous proteases in a manner which is analogous to the decrease in molecular rigidity and increase in susceptibility to calpain upon dephosphorylation of tau (23, 35). Finally, one of the viral proteases itself might have a limited activity on MAP 4. Purified retrovirus proteases have been shown to degrade MAPs in vitro (50), and thus poliovirus protease 2A or 3C might have a similar activity.

Since the alteration in MAP 4 occurs late after infection, it might not be expected to be involved in early steps in the replication cycle unless local changes resulting in cleavage of only a minor portion of the protein in the cell are sufficient for such a function. Late virus-specific functions which are more likely to be affected by cytoskeletal changes include packaging, movement of vesicles containing newly synthesized virions to the cell surface, and release of progeny virus

from the cell. Further studies will be necessary to determine the viral mediator of MAP 4 cleavage, the possible role of signal transduction mechanisms, and the effect of cleavage on microtubules, as well as any possible role of the MAP 4 alteration in viral replication.

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