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Human Cytomegalovirus-Induced Immediate Early Antigens: Analysis in Sodium Dodecyl Sulfate-Polyacrylamide Gel Electrophoresis After Immunoprecipitation

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Immediate early antigen (IEA) induced in human lung fibroblasts by human cytomegalovirus was characterized by sodium dodecyl sulfate-polyacrylamide gel electrophoresis analysis after immunoprecipitation with IEA-positive human sera. Two polypeptides of 76,000 daltons (76K) and 82K were detectable within 90 min after infection. Polypeptides of similar molecular weight were also found in immunoprecipitates of human cytomegalovirus-infected cells nonpermissive for virus replication. IEA is located within the nucleus, although some of the 76K material appears to be located on the outer nuclear membrane. Raising salt concentrations in the extraction buffer increased antigen extraction. The contribution of these IEA polypeptides to IEA nuclear fluorescent staining is discussed.

Using indirect immunofluorescence (IIF) and human sera, human cytomegalovirus (HCMV)-induced antigens have been divided into two main groups, "early" and "late," in the function of their appearance before or after viral DNA synthesis, respectively. Among the early antigens, the following have been described: immediate early antigen (IEA) (13, 15), early nuclear antigen (4, 5, 21, 22), early cytoplasmic antigen (22), and early membrane antigen (1). IEAs are detected by IIF within 20 min after infection as homogeneous nuclear fluorescence (13, 15). Early antigens, as defined by The et al. (21), are nuclear antigens detected by IIF in cells arrested by cytosine arabinoside for 72 h after infection and, therefore, represent an accumulation of virus-induced products appearing before the synthesis of viral DNA. Late antigens correspond to the fluorescent staining of the nuclear inclusion body and appear in cells after viral DNA synthesis takes place.

As yet no correlations have been made between the protein composition of early immunofluorescent antigens and HCMV-induced infected-cell-specific proteins (ICSPs) (16, 17). Knowledge of such a relationship is necessary to understand the role of such antigens in virus-cell relations and virus replication.

Among ICSPs the immediate early proteins are of special interest in that (i) their induction appears necessary for continuation of the viral cycle (17); (ii) these proteins are probably involved in chromatin template activation (7); and (iii) they appear in cells nonpermissive for virus replication (13, 15). For these reasons, we have investigated the polypeptide composition of ICSPs participating in IEA fluorescence.

The results of a characterization of IEA on sodium dodecyl sulfate-polyacrylamide gel electrophoresis (SDS-PAGE) after immunoprecipitation with human sera, using techniques successfully applied to the characterization of simian virus 40 T antigen (9), are reported and discussed herein.

MATERIALS AND METHODS

Buffers. (i) Extraction buffer (EB) is composed of 0.02 M Tris-hydrochloride (pH 9), 0.3 M NaCl, 10% (vol/vol) glycerol, 0.001 M $CaCl_2$, 0.0005 M $MgCl_2$, 0.002 M EDTA, and 0.5% (vol/vol) Nonidet P-40 (NP-40). (ii) Reticulocyte standard buffer consists of 0.01 M NaCl-0.01 M Tris-hydrochloride (pH 7.2)-0.015 M $MgCl_2$. EB and reticulocyte standard buffer both contained the following inhibitors of protease: 0.2 mM phenylmethylsulfonyl fluoride and 0.1 mM diisopropylfluorophosphate. (iii) Washing buffer contains 0.1 M Tris-hydrochloride (pH 9)-0.5 M lithium chloride-1% (vol/vol) beta-2-mercaptoethanol. (iv) Detaching buffer is 0.0625 M Tris-hydrochloride (pH 6.7)-2% (vol/vol) SDS-5% (vol/vol) beta-2-mercaptoethanol-15% (vol/vol) glycerol-0.001% (vol/vol) bromophenol blue. (v) Protein A buffer consists of 0.025 M Tris-hydrochloride (pH 7.5)-0.02% (vol/vol) sodium azide. (vi) Electrophoresis migrating buffer is composed of 0.025 M Tris-hydrochloride (pH 8.3)-0.192 M glycine-0.1% (vol/vol) SDS. (vii) Stacking gel buffer is 0.125

M Tris-hydrochloride (pH 6.8)–0.1% (vol/vol) SDS. (viii) Running gel buffer contains 0.375 M Tris-hydrochloride (pH 6.8)–0.1% (vol/vol) SDS. (ix) HEPES buffer consists of 25 mM HEPES–0.001 M $MgCl_2$–0.0005 M $CaCl_2$ (pH 6.8).

Cells. MRC-5 (6), human diploid fibroblasts (passages 26 to 35), and the rabbit skin fibroblast cell line RSB-1 (isolated in our laboratory by M. L. Ryhiner) were grown and maintained in antibiotic-free Eagle basic medium with 10% aseptic calf serum (Medical and Veterinary Supplies, Slough, England), 2% TRICINE (1 M) buffer, and 1.10 g of bicarbonate per liter. Labeling of cells was carried out in minimum essential medium without methionine containing 2% TRICINE buffer and antibiotics (50 μg of streptomycin and 200 IU of penicillin per ml). During chase periods, cells were maintained in Leibovitz L-15 medium with 5% calf serum, antibiotics, and 2% TRICINE.

Virus. The Mira strain (12) of HCMV was isolated some 15 years ago by A. Boué (Centre International de l'Enfance, Paris, France) from the blood of a newborn child who succumbed to severe generalized disease. Before entering our laboratory, the virus was passaged 20 times by transfer of infected cells to uninfected monolayers of WI-38 cells. Since then, it has been passaged as a cell-free inoculum in human lung fibroblasts 50 times and was used here at passages 37 to 46.

Isotopes. [^{35}S]methionine (specific activity, 700 Ci/mmol) was purchased from the C.E.A. at Saclay, France, or from The Radiochemical Centre, Amersham, England. Cells were labeled in suspension, after trypsinization and washing, with 50 μCi of [^{35}S]methionine per ml.

Sera. This work was done using the sera listed in Table 1.

Antigen preparation. Unless otherwise indicated, antigen was prepared from 5×10^6 cells 3 h after infection with 1 PFU of strain Mira HCMV per cell. After labeling, cells were washed two times in ice-cold phosphate-buffered saline containing Ca^{2+} and Mg^{2+} and the protease inhibitors phenylmethylsulfonyl fluoride plus diisopropylfluorophosphate. Antigen was then extracted by resuspending cells in EB and allowing them to stand for 20 min in an ice bath. Nuclei were sedimented off at 800 × *g* for 4 min at 4°C, and the resulting supernatant was further centrifuged for 30 min at 30,000 × *g* at 4°C to remove cell debris. Antisera (5 μl) were then added, followed by the addition of 10 times the volume of antisera of protein A-Sepharose CL-4B (Pharmacia) (a 50% solution = 1 volume of swollen gel per liter of protein A buffer). The final volume of antigen before the addition of serum and protein A was 0.5 ml. These mixtures were agitated vigorously overnight at 4°C. Sepharose–protein A-bound immunoprecipitates were washed by filtration on linear polyethylene (Bel Art) filters, using 2× 0.2 ml and 1× 0.5 ml of washing buffer at room temperature. Antigen-antibody complexes were then dissociated, using 3× 30 μl of detaching buffer held at 50°C. Each application of detaching buffer was made at a 10-min interval. Radioactivity in antigen preparations and in immunoprecipitates was precipitated with 5% trichloroacetic acid at 4°C and counted in a PPO-POPOP [2,5-diphenyloxazole–1,4-bis-(5-phenyloxazolyl)benzene] toluene-based scintillation fluid.

TABLE 1. *Characteristics of human sera used for immunoprecipitation*

Serum	IIF[a] antibody titers to:		
	IEA[b]	EA[c]	LA[d]
M19	1,600	2,500	360
HO288-2	160	80	160
MAP 64	160	50	40
MAP 51	80	25	160
MAP 65	40	80	40
GAR	0	0	0

[a] IIF was carried out on human diploid lung fibroblasts grown in Leibovitz L-15 medium supplemented with 10% calf serum. Cells (2×10^5 per well) were plated onto glass cover slips (16 by 16 mm) placed in wells of 25-well Sterilin culture plates and infected with 1 PFU of the Mira strain of HCMV per cell.

[b] Cover slips were sampled 3 h p.i.

[c] EA, Early antigen. Cover slips were prepared by growing infected cells in the presence of cytosine arabinoside (20 μg/ml) for 3 days from the time of virus adsorption.

[d] LA, Late antigen. Cover slips were sampled 4 to 5 days p.i.

The percentage of immunoprecipitable activity was estimated by calculating the total counts per minute in the immunoprecipitate divided by the total counts per minute in the antigen preparation before precipitation.

SDS-PAGE analysis (10). Unless otherwise indicated, running gels of 7.5 and 10% and stacking gels of 4% were used with a bis-acrylamide concentration of 2.5% and cross-linked with *N,N,N',N'*-tetramethylethylenediamine (Koch Light). Samples were heated at 100°C for 5 min before being applied to the gels. Gels were migrated at 25 mA until the blue marker was 1 cm from the bottom of the gel (about 4 h). Autoradiography was performed using Kodirex film. For fluorography (2), performed when the counts of any one sample were less than 10,000 cpm, gels were exposed to Kodak RP Royal X-Omat film.

Standards of known molecular weight were run in each gel and consisted of beta-galactosidase (130,000), alpha-phosphorylase (94,000), bovine serum albumin (68,000), catalase (60,000), L-glutamic dehydrogenase (53,000), ovalbumin (43,000), aldolase (40,000), lactoglobulin (18,400), and cytochrome *c* (12,000); molecular weights were calculated by regression. Gels were stained with 0.0125% Coomassie brilliant blue in 5.4% glacial acetic acid, 41.4% methanol, and 53.2% distilled water (all volume for volume). Gels were destained in the same solution minus the Coomassie brilliant blue.

IIF. The technique for IIF has been described elsewhere (13). M19 antiserum was used at a dilution of 1/250 and other sera were used at a dilution of 1/20. Fluorescein isothiocyanate-conjugated anti-human immunoglobulin G (Behring) was used at a dilution of 1/200.

RESULTS

SDS-PAGE analysis of proteins after immunoprecipitation with IEA$^+$ serum. Cells were labeled with [^{35}S]methionine for 2 h after

the end of virus adsorption (1 h) and precipitated with IEA-positive (IEA^+) sera. In parallel, uninfected cells labeled for the same length of time were reacted with the same IEA^+ sera. Figure 1 shows that all IEA^+ sera precipitated a band at 76,000 (76K) in infected cells (columns a–e). In addition, four of five sera also precipitated at 82K. The IEA specificity of these two bands was deduced by comparing gels of infected-cell IEA^+ serum precipitates (columns a–e) with uninfected cells precipitated with the same sera (columns a′–e′). It can be seen that bands at 76K and 82K in infected cells may be considered as IEA ICSPs, since all other bands appear also in uninfected control cell precipitates.

To see if IEA ICSPs detected in human permissive fibroblasts were also induced in nonpermissive (rabbit skin) fibroblasts, nonpermissive and permissive cells were labeled from 1 to 3 h after infection with [^{35}S]methionine and precipitated with M19 IEA^+ serum (Fig. 2, columns c and d) and HCMV-negative serum (GAR) (columns b and e). These were compared with uninfected cells of the same types precipitated with M19 IEA^+ serum (columns a and f). Permissive and nonpermissive cells precipitated with M19 IEA^+ serum yielded bands at 76K and 82K. In RSB-1-infected cells two other bands were also visible (column c, Fig. 2). Because these bands were consistently present in RSB-1 cells and never present in human cells, their IEA specificity is questionable. No additional bands were evident in gels of 12%.

Immunoprecipitates represented, on the average, 0.45% of the total radioactivity in infected-cell extracts.

Control of extraction procedure for IEA from infected cells. To examine the effect of Tris buffer at alkaline pH in the presence of NP-40 (EB; see Materials and Methods) on the removal of IEA, IIF was performed on cover slip preparations of infected cells after treatment with EB, using techniques described by Tegtmeyer et al. (20). Three hours postinfection (p.i.) cells were incubated with EB for 20 min at 4°C before being fixed in methanol for fluorescence. As controls, similarly prepared cells were incubated with EB without NP-40 and EB at pH 6.8 with and without NP-40. Figure 3a shows that EB at pH 9 with NP-40 almost completely removes IEA fluorescence. The alkaline pH appeared to be critical since the same buffer at pH 6.8 with NP-40 did not significantly affect fluorescence (Fig. 3b).

The molarity of NaCl in the EB greatly affected the efficiency of antigen extraction. In-

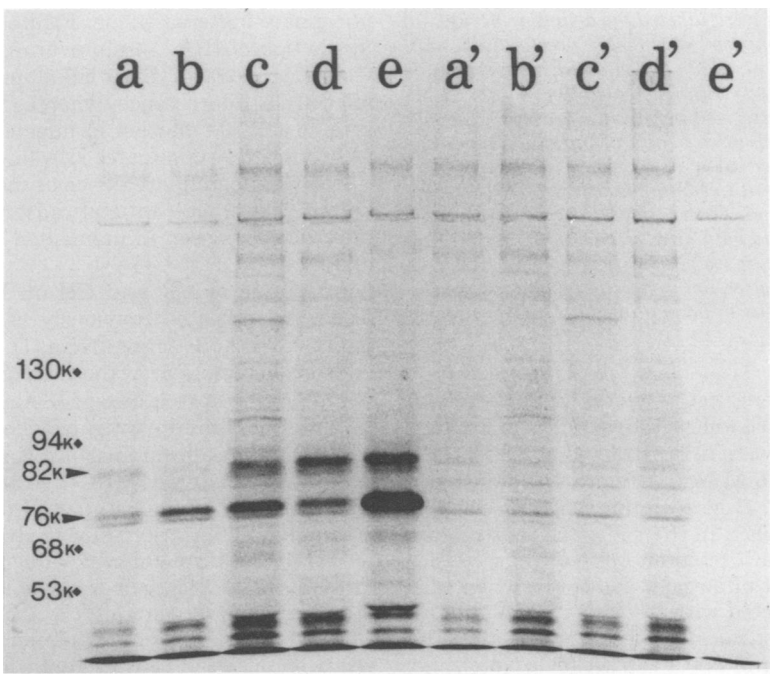

FIG. 1. *SDS-PAGE (7.5%) analysis of immunoprecipitates of cells with IEA^+ sera (a, a′ = 5 μl, M19; b, b′ = 10 μl, HO288-2; c, c′ = 10 μl, MAP 64; d, d′ = 10 μl, MAP 51; e, e′ = 10 μl, MAP 65). Infected cells (a–e) were labeled from 1 to 3 h after infection with [^{35}S]methionine. Uninfected cells (a′–e′) were labeled for the same length of time and precipitated with the same sera. Symbols: (◆) molecular weights of standard; (▶) molecular weights of IEA-specific bands calculated by regression (autoradiography).*

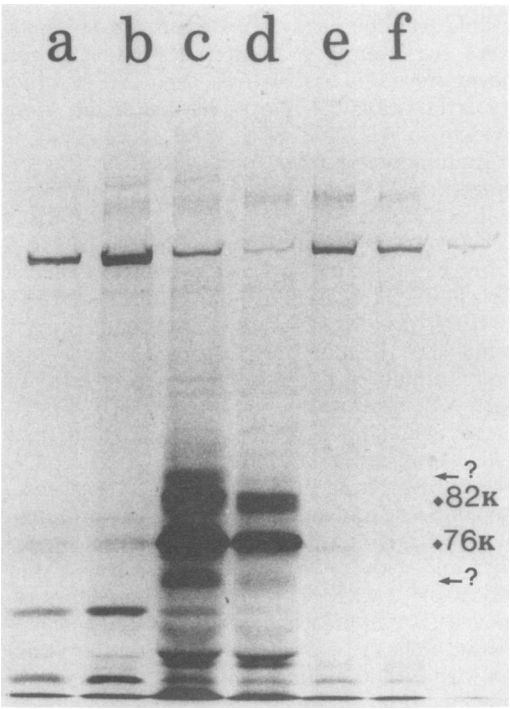

FIG. 2. *SDS-PAGE (7.5%) analysis of cells permissive (MRC-5) and nonpermissive (rabbit skin fibroblasts) for HCMV replication. Infected cells were labeled with [^{35}S]methionine from 1 to 3 h p.i. and uninfected cells were labeled for a similar period. (a) Uninfected, nonpermissive cells precipitated with IEA$^+$ serum (5 μl, M19); (b) infected, nonpermissive cells precipitated with IEA-negative serum (5 μl, GAR); (c) infected, nonpermissive cells precipitated with IEA$^+$ serum (5 μl, M19); (d) infected, permissive cells precipitated with IEA$^+$ serum (5 μl, M19); (e) infected, permissive cells precipitated with IEA-negative serum (5 μl, GAR); (f) uninfected, permissive cells precipitated with IEA$^+$ serum (5 μl, M19). Symbol: (◆) molecular weights of IEA-specific bands as calculated by regression in function of standards run in the same gel as indicated in Fig. 1 (autoradiography).*

fected cells were first extracted in the presence of 0.137 M NaCl followed by 0.6 M NaCl. Other infected cells were first extracted at 0.3 M NaCl followed by 0.6 M NaCl. Figure 4 shows that a molarity of 0.137 M (column a) leaves a major portion of antigen in the cell since reextraction with 0.6 M NaCl (column c) recovers a considerable amount of antigen. However, when cells are first extracted with 0.3 M NaCl (column b), there is almost no precipitable material in the subsequent 0.6 M NaCl extract (column e).

Kinetics of synthesis of IEA. Using IIF, it was found that IEA became visible 20 min after contact of cells with virus. Cells were therefore pulse-labeled with [^{35}S]methionine for 20, 50, 80, and 110 min after a 10-min virus adsorption period.

By immunoprecipitation (Fig. 5), the 76K and 82K ICSPs were first visible only 90 min after infection. At this time, the 82K band was less intense than the 76K band.

No precipitable material was seen in cells labeled for 3 h before infection and then chased for 3 h after infection.

Investigation of the role of IEA polypeptides in IEA immunofluorescence. In light of the fact that immunofluorescence detects IEA within 20 min after infection, whereas immunoprecipitation of labeled preparations detects material beginning 90 min p.i., it seemed of interest to investigate the participation of IEA polypeptides in immunofluorescence. For this purpose, antigen was prepared as above from 3-h p.i. cells (5×10^6). A 1/500 dilution of M19 IEA$^+$ serum was made directly in the antigen preparation and allowed to adsorb overnight at 4°C with vigorous shaking. As controls, 1/500 dilutions of the same IEA$^+$ serum were made in extracts from uninfected cells and in EB alone and adsorbed overnight. After adsorption, all preparations were centrifuged for 30 min at 12,000 × *g* and 4°C. Each serum thus adsorbed was applied to both uninfected and 3-h p.i. infected cells as antisera in IIF. As an additional control the same IEA$^+$ serum was freshly diluted 1/500 in phosphate-buffered saline. Figure 6 shows that incubation of IEA$^+$ serum overnight with uninfected cell extracts (B) or EB alone (G) does not affect IEA fluorescence, whereas incubation of sera at a 1/500 dilution in infected-cell extract (A) completely eliminates IEA fluorescence.

Early antigen fluorescence of the same serum persisted after IEA antibody adsorption. No fluorescence was seen in uninfected cells (Fig. 6E and F).

Influence of AD and CH on IEA appearance. As indicated previously (13), cycloheximide (CH) and actinomycin D (AD), when added 0.5 h before or at the time of infection, do not affect the appearance of IEA as detected by IIF. We therefore investigated the effect of CH and AD on the appearance of 76K and 82K IEA-precipitable material. MRC-5 cells were treated with either AD (5 μg/ml) or CH (50 μg/ml) for 30 min before infection, during virus adsorption (30 min), and throughout labeling with [^{35}S]methionine (2 h). Antigen was then extracted as described and precipitated with 5 μl of M19 IEA$^+$ serum. No immunoprecipitable material was seen in gels of cells treated with CH. Under these conditions [^{35}S]methionine incorporation was reduced by 97%. In immunoprecipitates of AD-treated cells, a faint band at 76K could be seen (Fig. 7). Under these conditions AD treat-

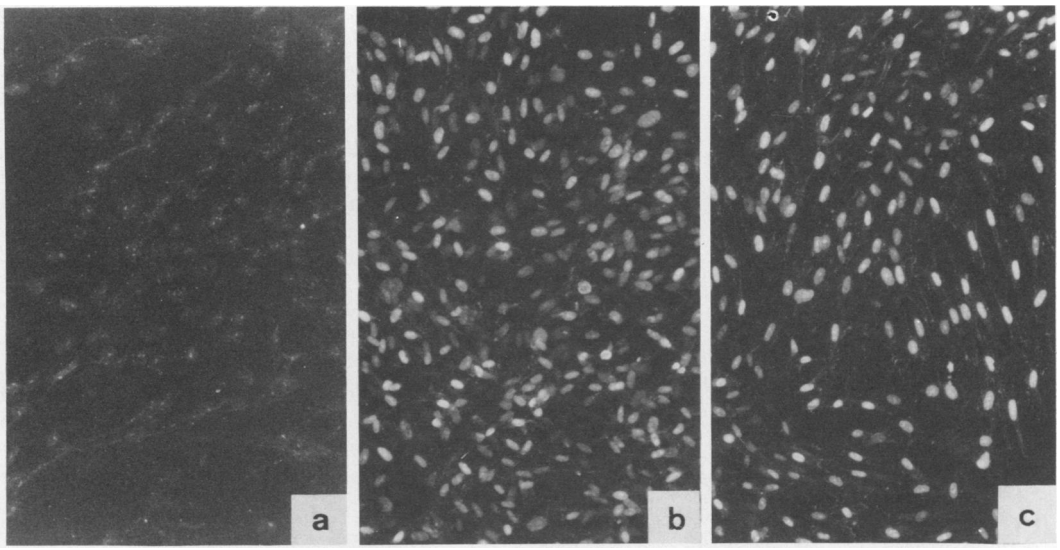

FIG. 3. *Effect of EB on the immunofluorescence of HCMV IEA. Cover slip preparations of MRC-5 human diploid fibroblasts were infected for 3 h and then treated with various buffers for 20 min at 4°C before immunofluorescence was performed using IEA$^+$ serum (M19). (a) Lack of fluorescence when treated with EB at pH 9 with 0.5% NP-40. (b) Positive fluorescence when treated with EB at pH 6.8 with 0.5% NP-40. (c) Control of positive fluorescence after incubation with phosphate-buffered saline + Ca^{2+} + Mg^{2+}.*

ment reduced [^{3}H]uridine incorporation by 94% and [^{35}S]methionine by 50%.

To determine if inhibitor-treated cells could adsorb out IEA antibodies, MRC-5 cells (5×10^6) were treated with AD (5 μg/ml) or CH (50 μg/ml) for 30 min before infection, during virus adsorption (30 min), and for 2 h thereafter. Antigens were extracted as described in Materials and Methods, and M19 IEA$^+$ serum was diluted 1/500 directly in these antigen extracts. After adsorption overnight at 4°C with vigorous shaking, samples were centrifuged at 12,000 × *g* for 30 min at 4°C. Each adsorbed serum was then used as antiserum in the first stage of immunofluorescence on cells 3 h p.i.

Adsorption by AD- and CH-treated infected cells failed to remove the fluorescent staining of IEA when adsorbed sera were tested on cells which had been infected for 3 h (Fig. 6A and C). These results were predictable in light of the small, or nonexistent, amounts of IEA ICSPs synthesized in the presence of these inhibitors as seen in SDS-PAGE gels after immunoprecipitation.

Localization of IEA within the cell. After being labeled and washed in phosphate-buffered saline (see Materials and Methods), half of the cells were allowed to swell for 10 min at 4°C in and reticulocyte standard buffer and then were subjected to 12 strokes in a Dounce homogenizer fitted with piston B. The other half of the cells were allowed to swell in reticulocyte standard buffer with 0.5% NP-40 for 10 min before being rapidly mixed in a Vortex mixer. Nuclei were centrifuged off at 800 × *g* for 4 min and resuspended in EB for 20 min to extract antigen. Cytoplasmic fractions were centrifuged further at 30,000 × *g* for 30 min at 4°C and then diluted 1/1 with EB; the concentration of NP-40 was adjusted to 0.5% to extract antigens. All fractions were precipitated with 5 μl of M19 IEA$^+$ serum overnight at 4°C with shaking and analyzed on 10% SDS-PAGE. In the presence of NP-40, the 76K band appears in both cytoplasmic (column c) and nuclear (column a) fractions (Fig. 8). The 82K band was only present in the nuclear fraction (column a), where it was substantially reduced. In the absence of NP-40, all precipitable material segregated with the nucleus (column b).

DISCUSSION

Much attention has recently been given to events occurring immediately after infection of cells with HCMV, notably because of virus-induced stimulation of host-cell functions (3, 18, 19). Kierszenbaum and Huang (8) showed that the ultrastructure of chromatin from infected cells displays novel bipartite and oblate ellipsoid structures. Kamata et al. (7) demonstrated that at 1 h p.i. two virus-induced proteins appear, one responsible for changes in chromatin template activity and another for the modification to

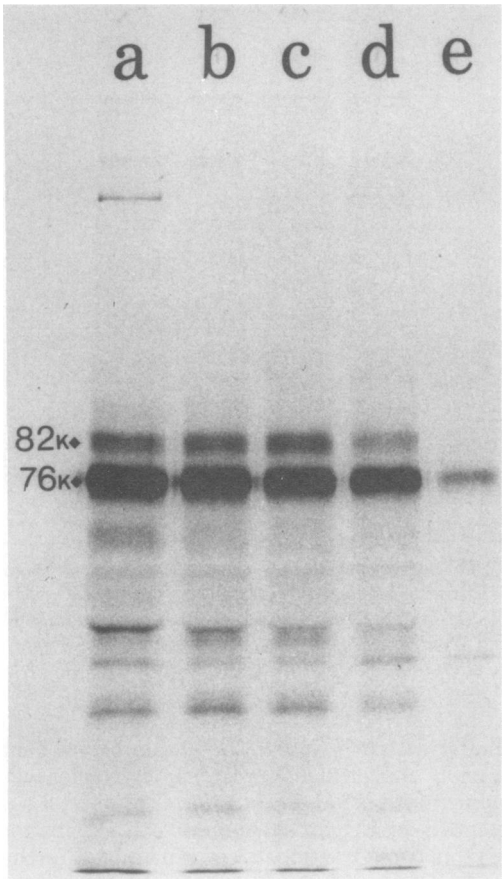

FIG. 4. *Efficiency of IEA extraction as a function of NaCl concentration in EB as revealed by SDS-PAGE (10%) analysis of immunoprecipitates using IEA$^+$ serum (5 μl, M19). Infected cells were labeled with [^{35}S]methionine from 1 to 3 h p.i., and antigen was extracted with buffer containing (a) 0.137 M NaCl, (b) 0.3 M NaCl, or (c) 0.6 M NaCl. (d) Residual antigen recovered by extraction at 0.6 M NaCl of cells previously extracted with 0.137 M NaCl. (e) Residual antigen recovered by extraction at 0.6 M of cells previously extracted at 0.3 M NaCl. Symbol: (◆) molecular weights of IEA-specific bands calculated by regression as a function of known standards run in the same gel (autoradiography).*

chromatin structure. Study of such immediate early events is important to understand virus-cell relationships.

Earlier, we showed by IIF the appearance of IEA (13), which has been confirmed by others (15). It is important to identify these virus-induced antigens since they probably represent the first viral polypeptides synthesized in infected cells. To identify them, we took advantage of techniques developed for studying simian virus 40 T antigen, notably, immunoprecipitation (9). Using this technique, we found that only two IEA ICSPs are consistently present in the nuclei of infected permissive and nonpermissive cells. These have molecular weights of 76K and 82K. The main arguments in favor of these being virally induced proteins are: (i) using IEA$^+$ sera, these polypeptides are only present in infected cells and are absent from uninfected cells; (ii) both polypeptides are precipitated by a variety of HCMV-positive IEA$^+$, but not by IEA-negative, sera; (iii) both polypeptides are present in permissive as well as nonpermissive cells.

Stinski (17) has reported that 6 h after infection three ICSPs (molecular weights of 75K, 72K, and 68K) can be detected using CH-treated cells and hypertonic medium. In light of the differences in experimental approach, it is difficult to superimpose our results on his. It can be

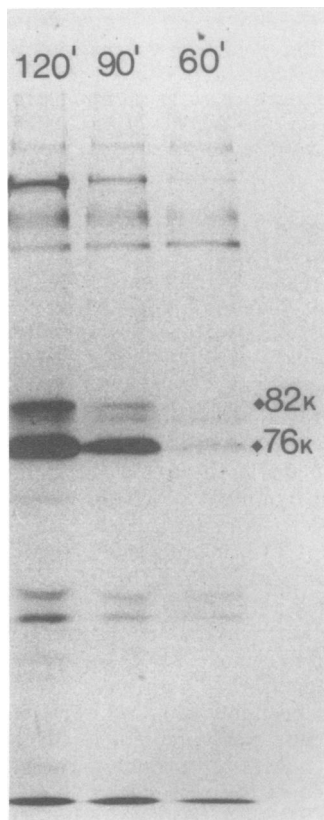

FIG. 5. *Kinetics of appearance of IEA in infected cells as revealed by SDS-PAGE (10%) analysis. Virus was adsorbed to cells for 10 min, and then cells were labeled with [^{35}S]methionine until (c) 60 min p.i., (b) 90 min p.i., and (a) 120 min p.i. Antigen was then extracted and precipitated with IEA$^+$ serum (5 μl, M19). Symbol: (◆) molecular weights of IEA-specific bands calculated by regression as a function of known standards run in the same gel (fluorography).*

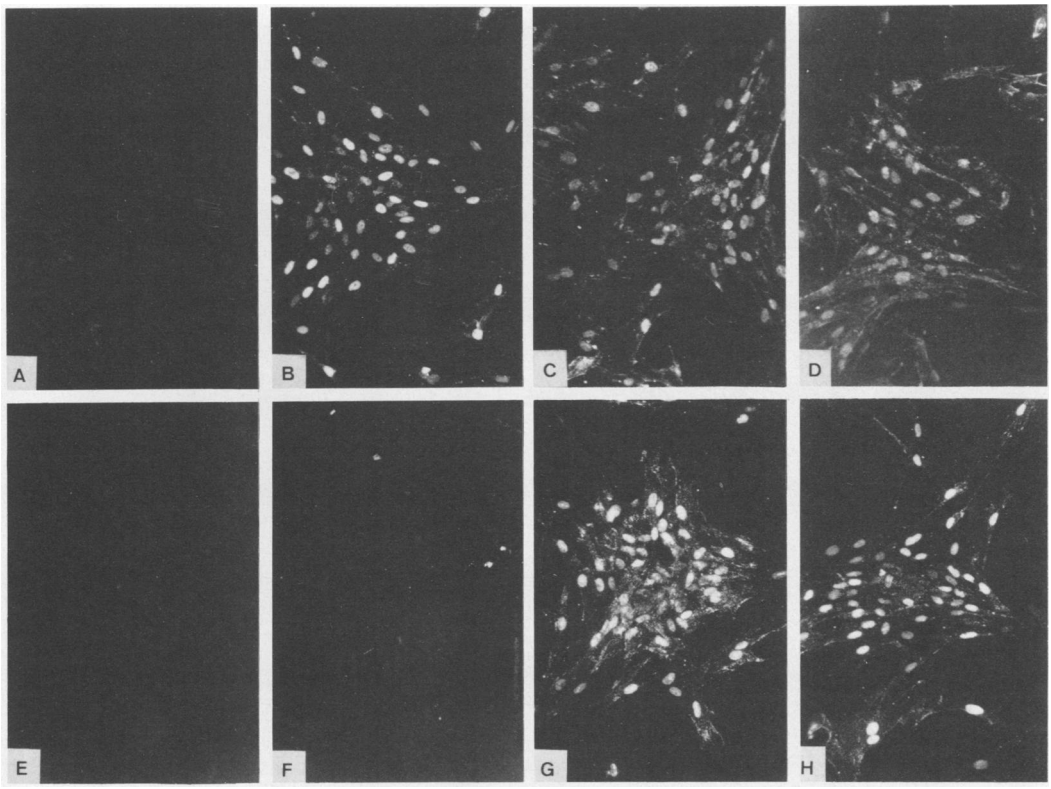

FIG. 6. *IEA immunofluorescence obtained with M19 IEA$^+$ serum adsorbed with antigen extracts from infected and uninfected cells. Two microliters of M19 IEA$^+$ serum was added to 1 ml of antigens extracted from 5 × 10^6 MRC-5 human diploid fibroblasts which had been infected for 3 h or left uninfected. Adsorption was carried out overnight at 4°C. After centrifugation, supernatants were used in IIF to detect IEA. IEA fluorescence in infected cells 3 h p.i. using M19 IEA$^+$ serum adsorbed with: (A) IEA antigen; (B) uninfected cell extract; (C) IEA extracted from cells blocked with AD (5 μg/ml); (D) IEA extracted from cells blocked with CH (50 μg/ml). Uninfected cell fluorescence using M19 IEA$^+$ serum adsorbed with: (E) IEA extracted from cells blocked with AD; (F) IEA extracted from cells blocked with CH. IEA fluorescence in infected cells using: (G) a 1/500 dilution of M19 IEA$^+$ serum made in EB and incubated overnight at 4°C; (H) a 1/500 dilution of M19 IEA$^+$ serum diluted in phosphate-buffered saline.*

said, however, that the range of molecular weights is the same in both studies.

Experimental evidence presented here favors the conclusion that the 76K and 82K IEA polypeptides contribute in part to IEA fluorescent staining. Extraction techniques used for antigen preparation effectively removed IEA fluorescence, as did adsorption of IEA$^+$ sera with antigen preparations. Therefore, immunofluorescence and immunoprecipitation of IEA probably involve some of the same proteins. The persistence of fluorescence in cells treated with CH, yet the lack of immunoprecipitable material in antigen extracts of such cells and the fact that antigen extracts of CH- and AD-treated cells could not adsorb out fluorescent antibodies from IEA$^+$ serum, suggest that IIF is either positive with much smaller amounts of 76K and 82K polypeptides or that additional proteins not detectable by immunoprecipitation due to their low concentration are involved.

Study of the kinetics of the appearance of IEA showed that by IIF antigen is detected 20 min p.i., whereas it is not seen until 90 min p.i. in gels after immunoprecipitation. This discrepancy may be due to inherent differences in sensitivity of each method. Nonetheless, at least one virus function is expressed within 90 min and is due to de novo synthesis, since no immunoprecipitable material was found in cells labeled before infection.

The importance of these immediate early polypeptides in the infectious cycle in vivo can be inferred from the fact that over 40% of normal blood donors react to IEA (11). Reactivity to IEA correlates highly with active virus excretion

(14). Preliminary serological data from our laboratory (work done in collaboration with N. Cabau and A. Boué of the Centre International de l'Enfance, Paris) suggest that anti-IEA formation can persist from many months in asymptomatic non-immunosuppressed subjects. These findings raise the question of the significance of persistent anti-IEA formation. It is hoped that characterization and sequencing of the other HCMV-induced antigens may shed some light on this problem.

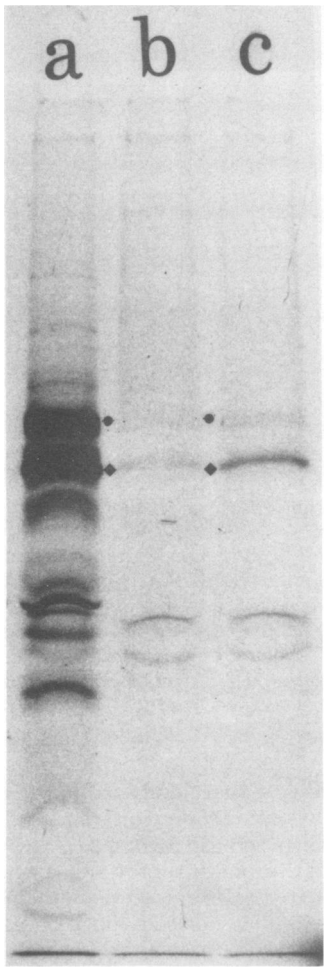

FIG. 7. *Effect of AD on the synthesis of HCMV IEA as revealed by SDS-PAGE (10%) analysis. Cells were infected and maintained in the presence of AD during labeling with [^{35}S]methionine from 1 to 3 h p.i. Similarly labeled, infected cells (a) were left untreated. All antigen extracts were precipitated with IEA$^+$ serum (5 μl, M19). (b) Cells receiving 10 μg of AD per ml; (c) cells receiving 5 μg of AD per ml. Faint bands can be seen at 76K (◆), whereas material is hardly visible at 82K (●). Molecular weights were calculated by regression as a function of standards run in the same gel (autoradiography).*

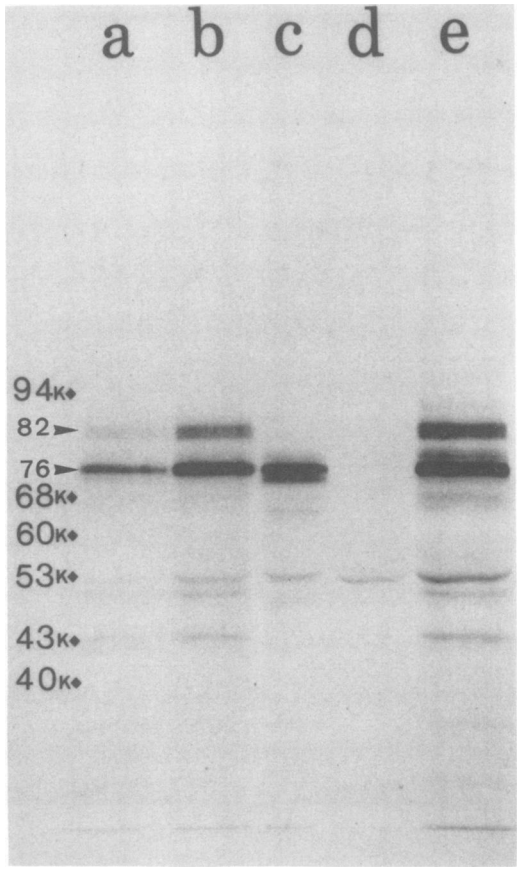

FIG. 8. *SDS-PAGE analysis of HCMV IEA extracted from nuclear and cytoplasmic fractions of infected cells. Cells, labeled with [^{35}S]methionine from 1 to 3 h p.i., were fractionated in reticulocyte standard buffer with and without 0.5% NP-40. All preparations were precipitated with IEA$^+$ serum (5 μl, M19). Nuclear fractions obtained in the (a) presence and (b) absence of NP-40. Corresponding cytoplasmic fractions obtained in the (c) presence and (d) absence of NP-40. A whole-cell extract was precipitated in parallel (e). Symbol: (◆) molecular weights of standards (autoradiography).*

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