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Intracellular Distribution of the Envelope Glycoprotein of Human Immunodeficiency Virus and Its Role in the Production of Cytopathic Effect in CD4$^+$ and CD4$^-$ Human Cell Lines

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Human CD4$^+$ and CD4$^-$ monocytoid cell lines were transfected with a constructed plasmid that has the envelope gene of human immunodeficiency virus under the transcriptional control of human metallothionein IIA promoter; the transfected cells were then cloned. These CD4$^+$ and CD4$^-$ transfectant cell clones, both of which expressed almost the same amount of gp160 after induction with metal ions, were used for ultrastructural analysis of the distribution of the envelope glycoprotein in the cytoplasm. Immunofluorescence microscopy with an anti-envelope glycoprotein monoclonal antibody showed localized distribution of gp160 in the CD4$^+$ cell clone and diffuse distribution of gp160 in the CD4$^-$ cell clone. These observations were substantiated by immunoelectron microscopy, in which the aggregated form of gp160 was observed in the cytoplasm of CD4$^+$ cells but was scarce in that of CD4$^-$ cells. A notable finding was that the sites corresponding to the nuclear pores were occupied with aggregates of gp160 in CD4$^+$ cells, exhibiting cytopathic effects. Both freeze-fracture and transmission electron microscopy also showed abnormal morphology around the nuclear pores and perinuclear space. These results support the possibility that such gp160 complexes accumulated around the nuclear pores primarily disturb the transportation of many molecules between the nucleus and the cytoplasm, resulting in a cytopathic effect in the CD4$^+$ cell clone.

Peripheral blood T lymphocytes or T-cell lines infected with human immunodeficiency virus (HIV) in vitro have been reported to produce dramatic cytopathic effects in their CD4-carrying cell populations (5, 9). The cellular basis by which HIV causes these cytopathic effects is not known. It has been proposed that cell death occurs primarily via an interaction between the viral envelope glycoprotein and the HIV receptor CD4 (6, 10, 15). This may lead to the formation of syncytia, causing cell death. However, the relatively infrequent occurrence of these fused cells in histopathological studies with specimens of postmortem acquired immunodeficiency syndrome patients (14) and an in vitro study showing cell death without any syncytium formation (16) suggested that cell fusion may not play a major role in HIV pathogenesis. On the other hand, a correlation seems to exist between the absolute level of CD4 molecule expression and the sensitivity of the host cell to viral cytopathic effects; T-cell lines, which express high levels of this receptor, are sensitive to cytopathic effects by HIV, whereas monocytoid cells, expressing a relatively low level of CD4 antigen, are resistant to such cytopathicity (1, 4). Asjö et al. (1) demonstrated that sensitivity to the cytopathic effects of HIV correlated with the expression level of CD4 antigen in U937 (a human monocytoid cell line) subpopulations. The U937 sublines that expressed a higher amount of CD4 were more sensitive than the sublines with a lower amount of CD4 to cytopathic effects by HIV. Furthermore, in superinfection of human T-cell leukemia virus type I-infected T-cell clones of either the CD4 or CD8 phenotype with HIV, a cytopathic effect was induced only in CD4$^+$ clones; the CD8$^+$ clone was resistant to the cytopathic effect (15).

HIV has several regulatory genes in addition to structural genes, enabling HIV to have a complicated life cycle in infected cells. Thus, in studies with HIV virions it is often difficult to draw a definitive conclusion concerning the role of each gene product in HIV pathogenesis. In a previous study (8), we constructed a plasmid, pSMTE7, that contains the *env* gene from the human T-cell leukemia virus type IIIB under the transcriptional control of the human metallothionein IIA promoter (7). A CD4$^+$ U937 subline, U937 clone 2, was then transfected with pSMTE7, and cells expressing the *env* gene after induction with metal ions were cloned. One of the cloned cell lines, EH, renamed U2ME7 in this report, expressed the viral glycoprotein gp160 in the cytoplasm but only barely on the cell surface after induction. The expression of surface CD4 antigen examined by anti-CD4 monoclonal antibodies disappeared completely in this cell line when gp160 was produced intracellularly. On the other hand, immunoprecipitation studies with lysate from these U2ME7 cells showed that OKT4 monoclonal antibody, which recognized the nonbinding site of CD4 for the HIV envelope glycoprotein, precipitated a significant number of CD4 molecules even after the surface CD4 disappeared. Moreover, Leu3a monoclonal antibody, which reacts with the binding site of CD4 for envelope glycoprotein, could not precipitate any CD4 molecules in the same sample of cell lysate. Taken together, these results implied that the gp160 molecules form a complex with the CD4 molecules in the cytoplasm, resulting in depletion of surface CD4 antigen, probably due to the disturbance in the transportation of CD4 molecules to the cell surface.

In the present study, the U937 parental cell line, which is a U937 cell subline bearing few CD4 molecules, was also transfected with pSMTE7. Another transfectant cell clone, UPME1, which expresses gp160 after induction with metal

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ions, was obtained. These $CD4^+$ and $CD4^-$ transfectant cell clones were used for ultrastructural analysis of the distribution of the envelope glycoprotein in the cytoplasm, and in this report we discuss the role of the envelope glycoprotein in the cytopathic effect in these cells.

MATERIALS AND METHODS

Establishment of transfectant cell clones that express the HIV *env* gene. Plasmid pSMTE7 (8) was constructed by inserting the promoter portion of the human metallothionein IIA gene and the sequence of the whole HIV *env* gene (BH10 clone, nucleotides 5496 through 8474) into plasmid pSV2neo, which possesses a gene encoding resistance to the antibiotic G418. The construct was transfected by the electroporation method (12) into human monocytoid cell sublines, U937 clone 2 ($CD4^+$) and the U937 parent ($CD4^-$). Flow cytometry and Northern RNA blot analyses showed significant expression of CD4 antigen and CD4 transcript in the U937 clone 2 but little expression in the U937 parent (Fig. 1). The cells were then selected for resistance to G418 and cloned. The isolated transfectant cell clones from U937 clone 2 and the U937 parent were tested for their expression level of *env* message by Northern blot analysis after the addition of 10 μM cadmium chlorides as an inducer. The cells were maintained at 37°C in RPMI 1640 medium supplemented with 10% fetal calf serum and antibiotics in humidified air containing 5% CO_2. The cell number was determined with a Coulter Counter (model ZB; Coulter Electronics, Inc., Luton, England).

Immunoblot analysis. For immunoblot analysis, cells were washed with phosphate-buffered saline (PBS) and lysed in a cold extraction buffer containing 20 mM Tris hydrochloride (pH 7.4), 0.15 M NaCl, 1% Triton X-100, 0.05% sodium dodecyl sulfate, and 2 mM phenylmethylsulfonyl fluoride. Proteins from each sample were separated by sodium dodecyl sulfate-polyacrylamide gel electrophoresis (7.5% acrylamide) under reducing conditions. The separated polypeptides were transferred to a nitrocellulose sheet and treated with an anti-HIV envelope glycoprotein monoclonal antibody (MAH-316; TOYOBO Co., Ltd., Osaka, Japan) in the reaction solution (5% skim milk, 0.5 M NaCl, 20 mM Tris hydrochloride [pH 7.4], 0.05% Tween 20) and ^{125}I-labeled protein A as an indicator of bound antibodies for visualization on X-ray film.

Immunofluorescence microscopy (IFM). An anti-envelope glycoprotein monoclonal antibody was added to cells with or without fixation by 75% ethanol and incubated for 45 min on ice. After the cells were washed twice with PBS, they were stained with fluorescein isothiocyanate-conjugated anti-mouse immunoglobulin G (IgG; Tago Inc., Burlingame, Calif.) and mounted for viewing with a fluorescence microscope. The levels of expression of CD4 on the cell surface were evaluated with a FACScan flow cytometer (Becton Dickinson Labware, Mountain View, Calif.).

Immunoelectron microscopy (IEM). Cells were fixed with 2% paraformaldehyde and 0.2% glutaraldehyde for 30 min and treated with 0.5 M $NaBH_4$. After the cells were washed with PBS, they were dehydrated with 50, 70, 90, and 100% ethanol serially at 4°C (50% ethanol) and −20°C (70, 90, and 100% ethanol). Then the cells were embedded in Lowicryl K4M and polymerized at −20°C by UV irradiation. Thin sections prepared from cell blocks were then placed on nickel grids. These grids were treated sequentially with reagents at room temperature in the following order: 1% bovine serum albumin–PBS; 100 μg of anti-envelope glycoprotein monoclonal antibody or OKT4 monoclonal antibody (Ortho Diagnostics, Inc., Raritan, N.J.) per ml for 12 h; 1% bovine serum albumin–PBS four times for 3 min each; colloidal gold particles coated with anti-mouse immunoglobulin antibody at 5 times dilution (product no. 2368618; Janssen Life Science Products, Olen, Belgium); PBS three times for 3 min each; 2.5% glutaraldehyde for 10 min; and distilled water once for 3 min. The thin sections were finally stained with uranyl acetate and lead citrate and viewed in a JEM-2000EX electron microscope (JEOL Ltd., Tokyo, Japan).

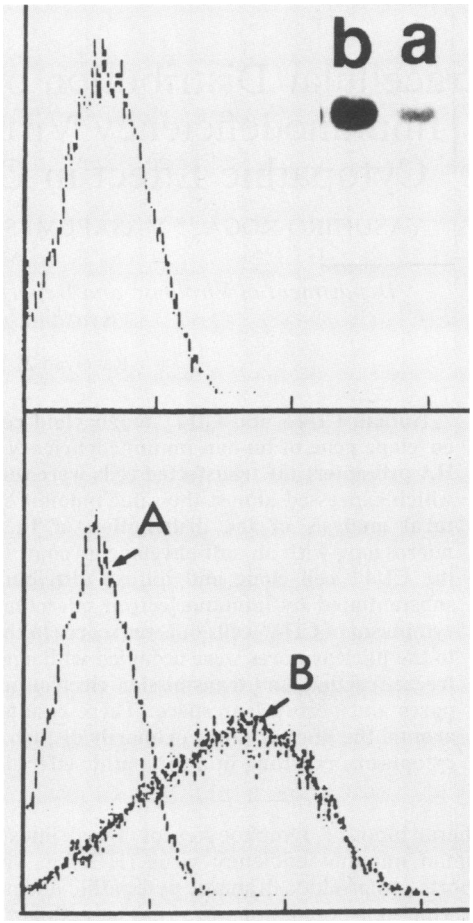

FIG. 1. Expression of the surface CD4 antigen and CD4 message. Expression levels of surface CD4 antigen were examined with a FACScan and fluorescein isothiocyanate-conjugated OKT4 monoclonal antibody in U937 parent (A) and U937 clone 2 (B) cells. The configuration in the upper panel shows the autofluorescence of these cells as the control. The upper panel also shows the message levels of CD4 transcripts in the U937 parent (a) and the U937 clone 2 (b) cells assayed by Northern blot analysis with human CD4 cDNA as described in our previous report (8).

Preparation for freeze-fracture replication. Cells suspended in PBS were fixed with 2× fix buffer (1× fix buffer is 2.5% glutaraldehyde, 0.1 M sucrose, and 3 mM $CaCl_2$ in 0.1 M cacodylate buffer [pH 7.4]) for 30 min at 37°C, pelleted, and soaked with 40% glycerol in 0.1 M cacodylate buffer for 2 h at 4°C. Then a small piece of material was placed on a specimen holder and frozen in liquid Freon. This frozen specimen was then fractured in a JFD7000 freeze-fracture and etching device (JEOL) at −180°C and at high vacuum (10^{-7} Torr). Replicas were made by rotary shadowing with a

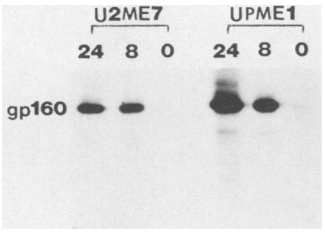

FIG. 2. Immunoblot analysis of expression of envelope glycoprotein in *env* gene-transfected cells. U2ME7 and UPME1 cells were incubated with 10 μM $CdCl_2$ for 0, 8, and 24 h. Cell lysates were prepared from the treated cells and loaded on a sodium dodecyl sulfate-polyacrylamide gel. The gel was electroblotted to a nitrocellulose membrane, and the filter was incubated with anti-envelope glycoprotein monoclonal antibody. Envelope glycoprotein was determined by binding of ^{125}I-labeled protein A.

mixture of platinum and carbon and viewed in a JEM-2000EX electron microscope.

Preparation for transmission electron microscopy (TEM). Cells (2×10^7) were suspended in 5 ml of PBS, fixed with 5 ml of 2× fix buffer, and kept in a fixative for 10 min at 37°C. After the cells were pelleted, they were processed through 1% OsO_4 for 30 min at 4°C, dehydrated in graded ethanol and propylene oxide, and embedded in Epon 812 resin. Thin sections (90 to 100 nm in thickness) were stained with 2% uranyl acetate for 60 min and lead acetate for 5 min and examined on a JEM-2000EX electron microscope at 80 to 100 kV.

RESULTS

Expression of HIV envelope glycoprotein in *env* gene-transfected cell clones. A $CD4^-$ human monocytoid cell line, the U937 parent, and its $CD4^+$subline, U937 clone 2, were transfected with pSMTE7, which expresses the HIV *env* gene under the transcriptional control of human metallothionein IIA promoter. Such transfected cells were screened and cloned with added G418. By using Northern blot and immunoblot analyses, U2ME7, derived from U937 clone 2, and UPME1, from the U937 parent, were finally selected and used in this study. Figure 2 shows an immunoblot analysis of envelope glycoprotein expressed in these cell clones after induction with 10 μM $CdCl_2$. The U2ME7 and UPME1 cells expressed almost the same amount of envelope glycoprotein at 8 and 24 h after induction. Although gp160 was easily detectable, the protein band corresponding to gp120 was difficult to detect, especially in U2ME7, even in an X-ray film subjected to longer exposure. To determine the distribution of envelope glycoprotein in the cells, IFM was performed with anti-envelope glycoprotein monoclonal antibody and fluorescein isothiocyanate-conjugated anti-mouse IgG. When membrane fluorescence was examined in viable cells, little or no fluorescence was detected on either U2ME7 or UPME1 cells before or after induction of gene expression (data not shown). Cell samples of parallel cultures were fixed with ethanol 8 h after induction to determine the intracellular distribution of the envelope glycoprotein by IFM (Fig. 3). U2ME7 cells (Fig. 3C), a $CD4^+$ transfectant cell clone, showed localized distribution of envelope glycoprotein in the cytoplasm, whereas UPME1 cells (Fig. 3D), a $CD4^-$ transfectant cell clone, exhibited diffuse distribution of gp160.

Ultrastructural analysis by IEM. To further examine the difference between U2ME7 and UPME1 cells with regard to intracellular distribution of envelope glycoprotein, IEM was

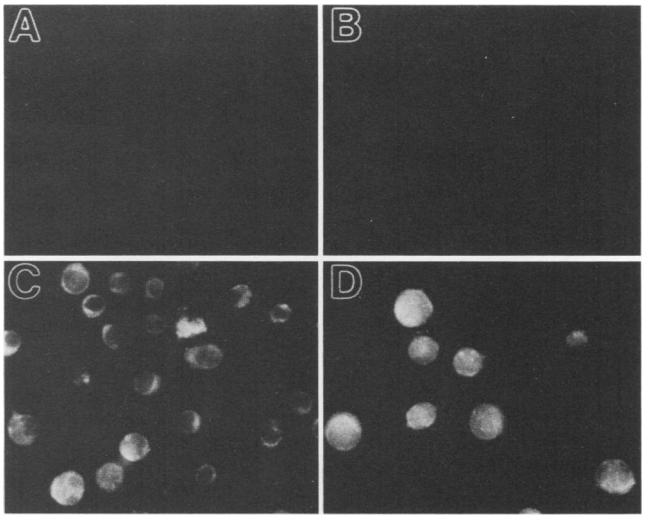

FIG. 3. IFM of envelope glycoprotein after induction. U2ME7 (C) and UPME1 (D) cells after treatment with 10 μM of $CdCl_2$ for 8 h were fixed with 75% ethanol and incubated with anti-envelope glycoprotein monoclonal antibody. The bound antibodies were then visualized by treating them with fluorescein isothiocyanate-conjugated anti-mouse IgG and examined with a fluorescence microscope. U2ME7 (A) and UPME1 (B) were treated with a standard mouse IgG and fluorescein isothiocyanate-conjugated anti-mouse IgG and thus represent background fluorescence patterns.

performed with mouse anti-envelope glycoprotein monoclonal antibody and colloidal gold particles coated with anti-mouse IgG (Fig. 4). Eight hours after induction, many aggregates consisting of more than 10 gold particles were found in the cytoplasm of the U2ME7 cells (Fig. 4B and D), whereas aggregates of gold particles were not found in the cytoplasm of UPME1 cells (Fig. 4A). The gold particles appeared to correspond to the localized form of the envelope glycoprotein found with IFM (Fig. 3). A notable finding in the IEM specimens was that most of the sites that seemed to be nuclear pores were labeled heavily by gold particles in many of the U2ME7 cells (Fig. 4B, C, and D). Moreover, IEM with OKT4 monoclonal antibody, which recognized the non-gp160/120 binding site of the CD4 molecule, also showed aggregates of gold particles at the nuclear pores in the serial specimens prepared from the same cell block for the detection of envelope glycoprotein (Fig. 4E). The accumulation of gold beads was not observed with NU-T3, a mouse monoclonal antibody for CD3, in this sample of U2ME7 (data not shown). Therefore, we supposed that the complex between the envelope glycoproteins and the CD4 molecules had accumulated around the nuclear pores in U2ME7 cells after induction.

Ultrastructural analysis by the freeze-fracture replication method. To investigate in more detail the changes in the nuclear membranes after induction, cells were analyzed by freeze-fracture replication (Fig. 5 and 6). Extensive deformities in the nuclear pores were observed in U2ME7 cells 8 h after induction (Fig. 5C and D). The deformities observed with the freeze-fracture method seemed to correspond to the accumulation of the envelope glycoprotein and/or CD4 molecule complex around the nuclear pores observed with IEM (Fig. 4). In addition, many irregular heaps were observed on the surface of the nuclear membrane (Fig. 5D). On the other hand, no significant changes occurred around the pores or

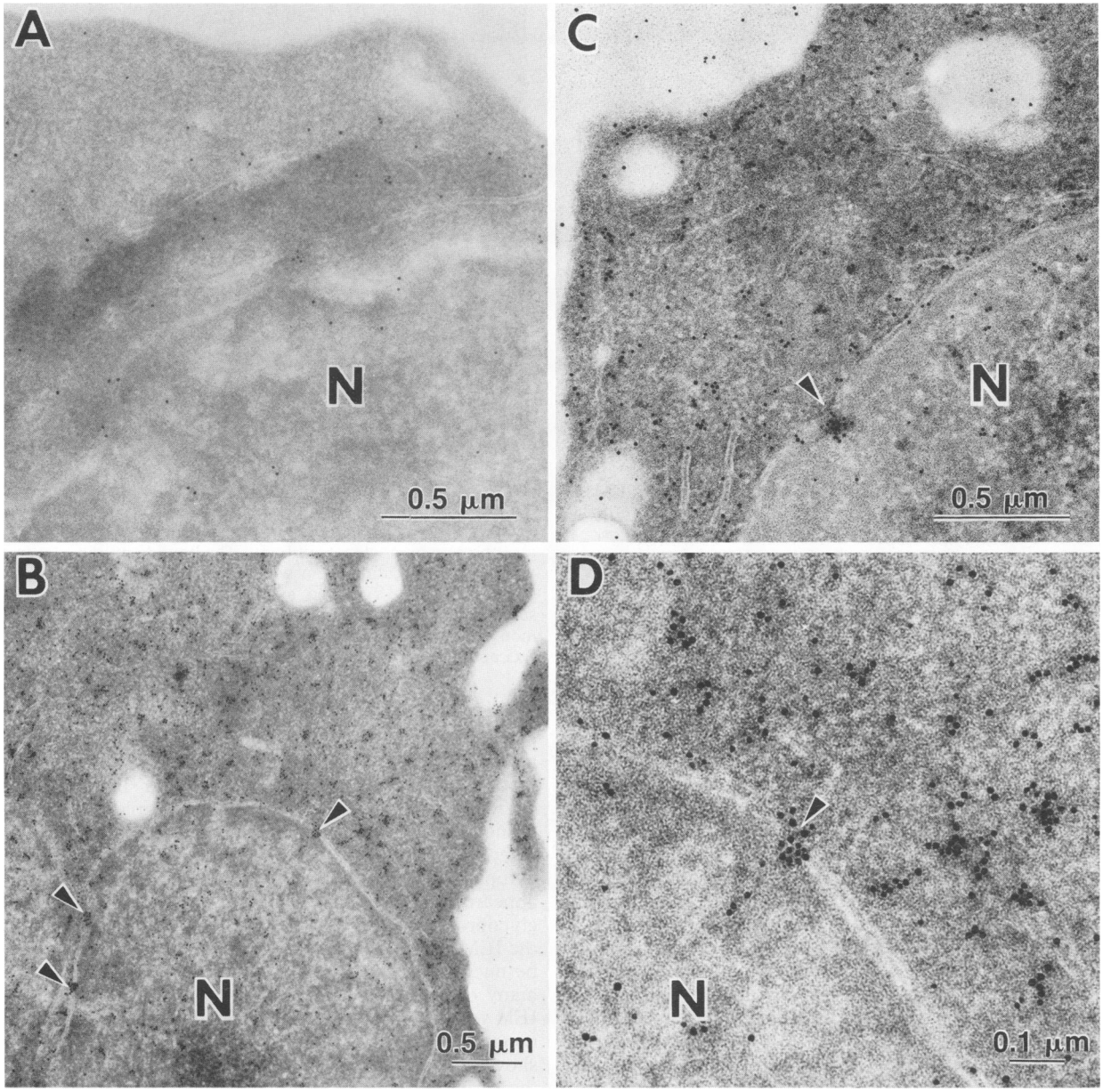

FIG. 4. IEM of envelope glycoprotein after induction. UPME1 (A) and U2ME7 (B, C, D) cells after treatment with 10 μM $CdCl_2$ for 8 h were examined by IEM with mouse anti-envelope glycoprotein monoclonal antibody and gold particles coated with anti-mouse immunoglobulin antibody. In panel B, 20 cells that contained the accumulated gold particles in the cytoplasm were picked up randomly for observation; 17 cells had aggregates of gold beads around their nuclear pores. Panel D is the higher magnification of panel B. (E) IEM of U2ME7 cells with OKT4 monoclonal antibody and the gold particles after induction. (F) IEM of the U2ME7 cells without cadmium induction with OKT4 and gold beads. The arrowheads indicate the accumulated gold particles around the nuclear pores except in the panel F, where there was no accumulation of gold particles. N, Nucleus.

the surface of nuclear membranes of UPME1 cells after induction (Fig. 6C and D).

Ultrastructural analysis with TEM. TEM analysis of ultrastructural morphology was performed in induced cells (Fig. 7 and 8). Eight hours after induction in U2ME7 cells, irregular enlargement of the perinuclear space between the outer and inner nuclear membranes was observed (Fig. 7D, E and F). Many vacuoles formed in the cytoplasm, and enlargements of the nucleolus were also found in this early stage of induction. These morphological alterations were not observed after induction of *env* gene in UPME1, which is derived from $CD4^-$ U937 (Fig. 8C and D). Therefore we concluded that morphological abnormalities, especially those of the nuclear membrane region in U2ME7 cells found with TEM, were caused by envelope glycoprotein and/or CD4 complex accumulated in the perinuclear region.

Different effects of *env* gene induction on the cell growth in $CD4^+$ and $CD4^-$ cells. The effect of intracellular production of envelope glycoprotein on cell growth was examined in U2M1 (which is transfected with pSMT and has the same structure as pSMTE7 except without the *env* insert), UPME1, and U2ME7 cells (Fig. 9). Each cell population was

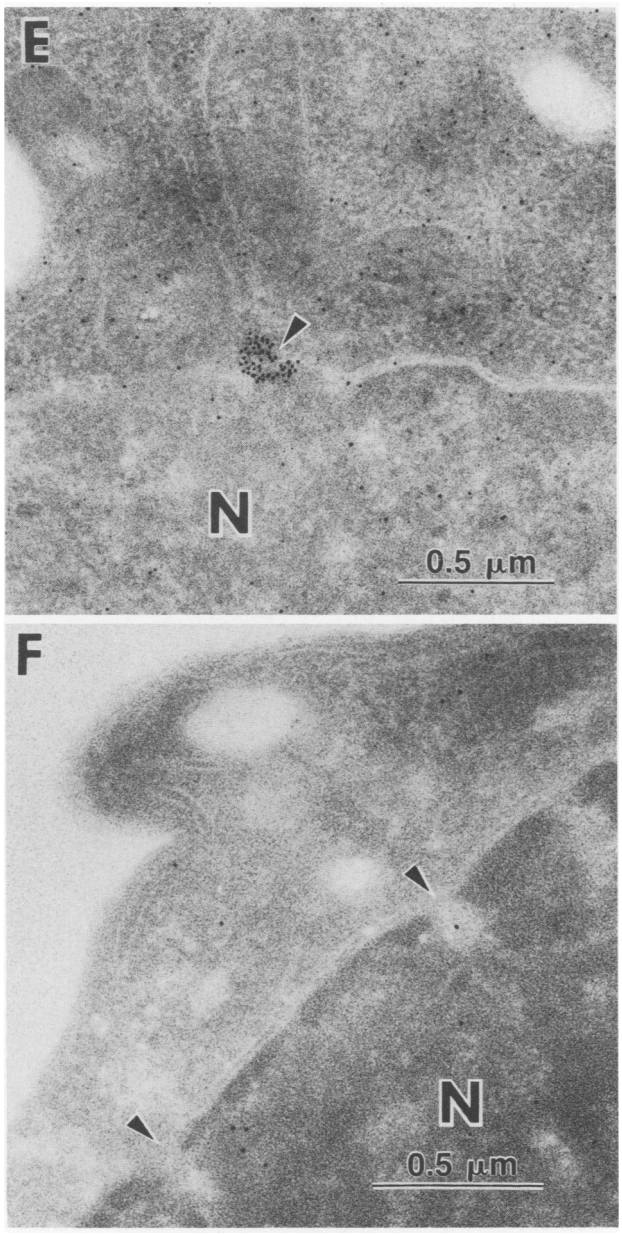

FIG. 4—*Continued*

adjusted to 10^5 cells per ml of RPMI 1640 medium supplemented with 10% fetal calf serum, and cell suspensions were put into 24-well dishes on day 0 without or with 10 μM $CdCl_2$ or 100 μM $ZnSO_4$ as the inducer. When expression of the *env* gene was induced, cell growth was blocked in U2ME7 ($CD4^+$ transfectant cell clone), whereas the growth of UPME1 ($CD4^-$ transfectant cell clone) was not affected by the expression of the *env* gene. In cultures of U2ME7 cells, many cells could not exclude the vital dye 2 days or later after induction, indicating that most of them were dead. An interesting finding was that each cell was isolated and did not form any syncytium in those cultures (data not shown). These results demonstrated that the cytopathic effect occurred only in the $CD4^+$ transfectant cell clone, although almost the same amount of envelope glycoprotein was produced in both the $CD4^+$ and $CD4^-$ transfectant cell clones, suggesting a significant role for CD4 molecules in cytopathic effects by formation of complexes with envelope glycoproteins in the cytoplasm.

DISCUSSION

The pathophysiology of acquired immunodeficiency syndrome is characterized by a gradual decrease in numbers of $CD4^+$ helper-inducer T cells, leading to a nearly complete failure to mount T helper cell-dependent immune responses to many types of infections (2). Most human monocytes express CD4 surface antigen and can bind to the envelope glycoprotein of HIV and thus are susceptible to HIV cytotoxicity (1, 4, 18). However, the precise mechanism of the cytopathic effect in $CD4^+$ cells infected with HIV still remains unknown. There is evidence that both the CD4

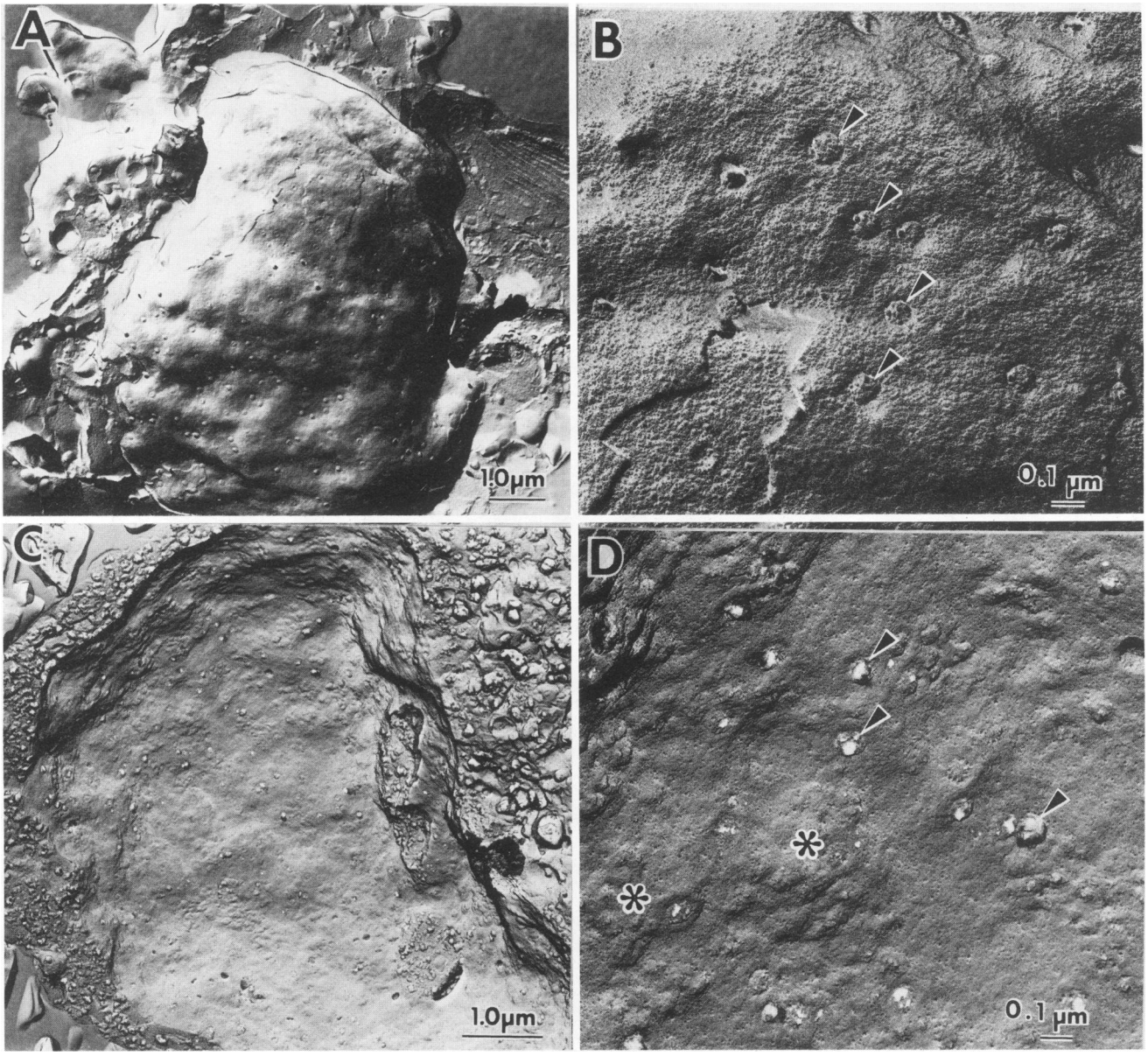

FIG. 5. Examination of the surface of nuclear membranes by the freeze-fracture method. U2ME7 cells were treated with 10 μM of $CdCl_2$, and their ultrastructure was examined by freeze-fracture at 0 h (A and B) and 8 h (C and D) after induction. Panels B and D are higher magnifications of panels A and C, respectively. The arrowheads and asterisks indicate nuclear pores and abnormal heaps of the nuclear membranes, respectively.

molecule and the virus envelope glycoprotein play a role in the cytopathic effect of infected $CD4^+$ cells (6, 10, 15). Asjö et al. (1) demonstrated that sensitivity to the cytopathic effect of HIV is correlated with the level of expression of the CD4 antigen in subpopulations of the U937 monocytoid cell line. In our previous study (8), we cloned a $CD4^+$ monocytoid cell line, U937 clone 2, that was transfected with a constructed plasmid that has the *env* gene of HIV under the transcriptional control of the human metallothionein IIA promoter. One of these cell clones, U2ME7, also used in this study, expressed viral gp160, which formed a complex with CD4 molecule in the cytoplasm. The surface expression of an envelope glycoprotein was almost undetectable in this cell clone. In the present study, another cell clone, UPME1, which expresses almost the same amount of gp160 as that of U2ME7, was prepared by transfecting pSMTE7 into the U937 parent, a $CD4^-$ U937 subline. In this cell clone also the surface envelope glycoprotein was almost undetectable. Asjö et al. (1) reported that the U937 clone 2, the same cell line used for transfection with the *env* gene in our study, was able to be infected with human T-cell leukemia virus type IIIB and produced gp120 as well as gp160. Thus, no expression of gp120 or surface membrane envelope glycoprotein in our system should be ascribed to the property of our *env* gene construct. With these $CD4^+$ and $CD4^-$ transfectant cell clones, the difference in the distribution of envelope glycoprotein was examined by morphological analysis. IFM with anti-envelope glycoprotein monoclonal antibody showed a localized distribution of the envelope glycoprotein in U2ME7 cells but diffuse distribution of the envelope glyco-

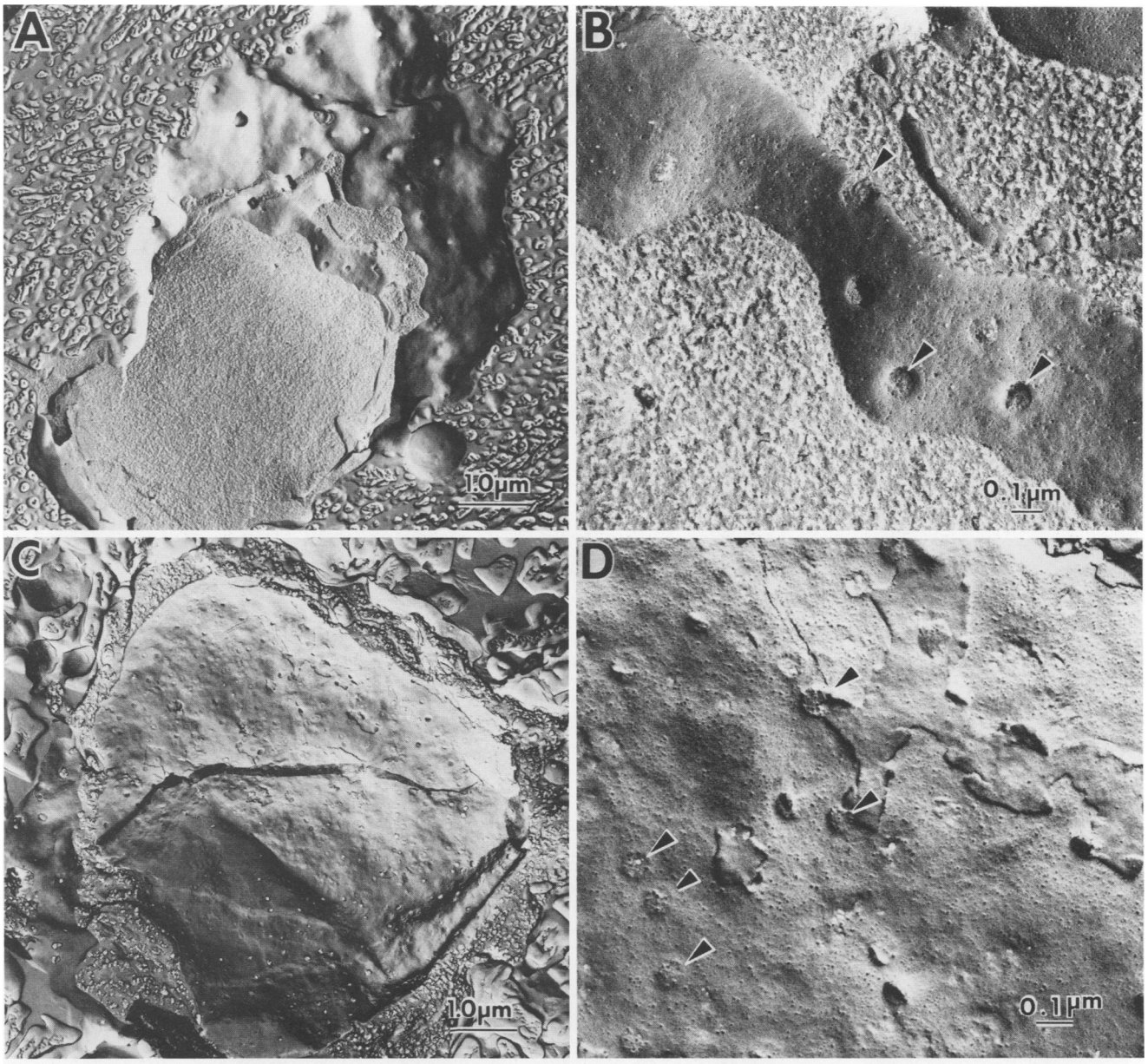

FIG. 6. Examination of the surface UPME1 cells by freeze-fracture. Panels and symbols are as described in the legend to Fig. 5.

protein in UPME1 cells. These observations were substantiated by immunocolloid gold electron microscopy with mouse anti-envelope glycoprotein monoclonal antibody and gold particles coated with anti-mouse immunoglobulin antibody; many aggregated gold particles were found in the cytoplasm of U2ME7 cells, but they were scarce in that of the UPME1 cells. Moreover, gold particles coated with OKT4 monoclonal antibody, which recognizes the non-envelope glycoprotein binding sites of the CD4 molecule, also accumulated in U2ME7 cells after induction. These results indicated that the gp160 molecules produced in U2ME7 cells are bound to CD4 molecules to form a large aggregate easily detected by IEM. The formation of such complexes between gp160 and CD4 in the cytoplasm of U2ME7 cells has already been confirmed by immunoprecipitation in our previous report (8). Moreover, with two-color IFM, the same patterns of distribution were observed in the cytoplasm of U2ME7 cells for both the envelope glycoprotein and the CD4 molecule, indicating the formation of an envelope-CD4 complex in the cytoplasm (data not shown). The absence of any aggregated forms of envelope glycoproteins in UPME1 cells in spite of the sufficient production of gp160 is considered to be due to the lack of CD4 molecule in these cells as the counterpart for such complexes.

A notable finding was that the sites corresponding to the nuclear pores were occupied with aggregates of gp160 and/or CD4 complex in U2ME7 cells. In addition, the freeze-fracture replication method showed an abnormal shape of nuclear pores. TEM analysis of these U2ME7 cells showed an enlargement of the perinuclear space between the outer and inner nuclear membranes, indicating the occurrence of some pathological events in the nuclear envelope. U2M1

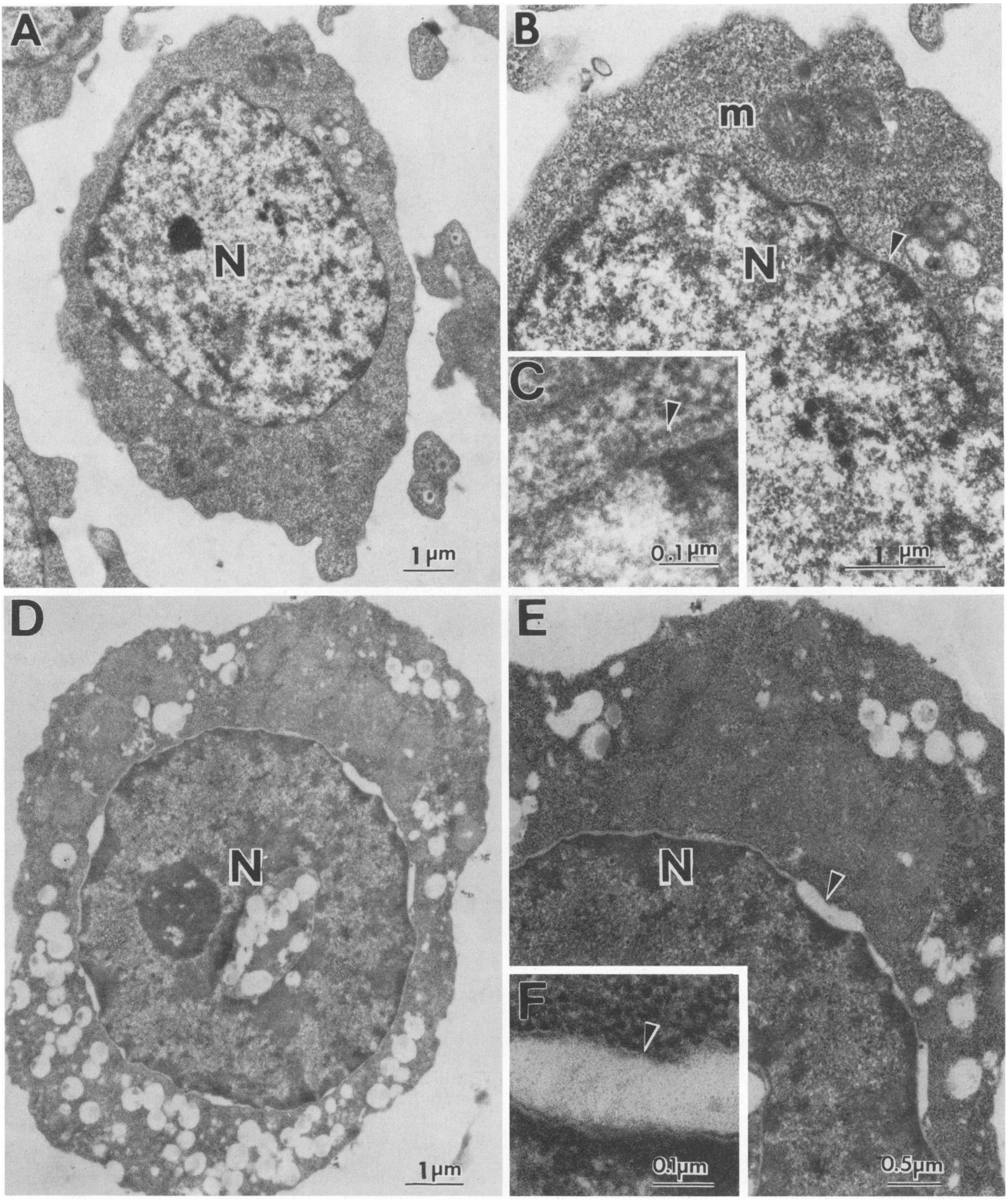

FIG. 7. Morphological analysis of U2ME7 cells by TEM. U2ME7 cells were treated with 10 μM $CdCl_2$ and sampled at 0 h (A, B, C) and 8 h (D, E, F) after induction. Samples were thin sectioned, and their ultrastructures were examined by TEM. Panels B and E are higher magnifications of panels A and D, respectively. Insets C and F are higher magnifications of the regions indicated by arrowheads in panels B and E, respectively. N, Nucleus; m, mitochondrion.

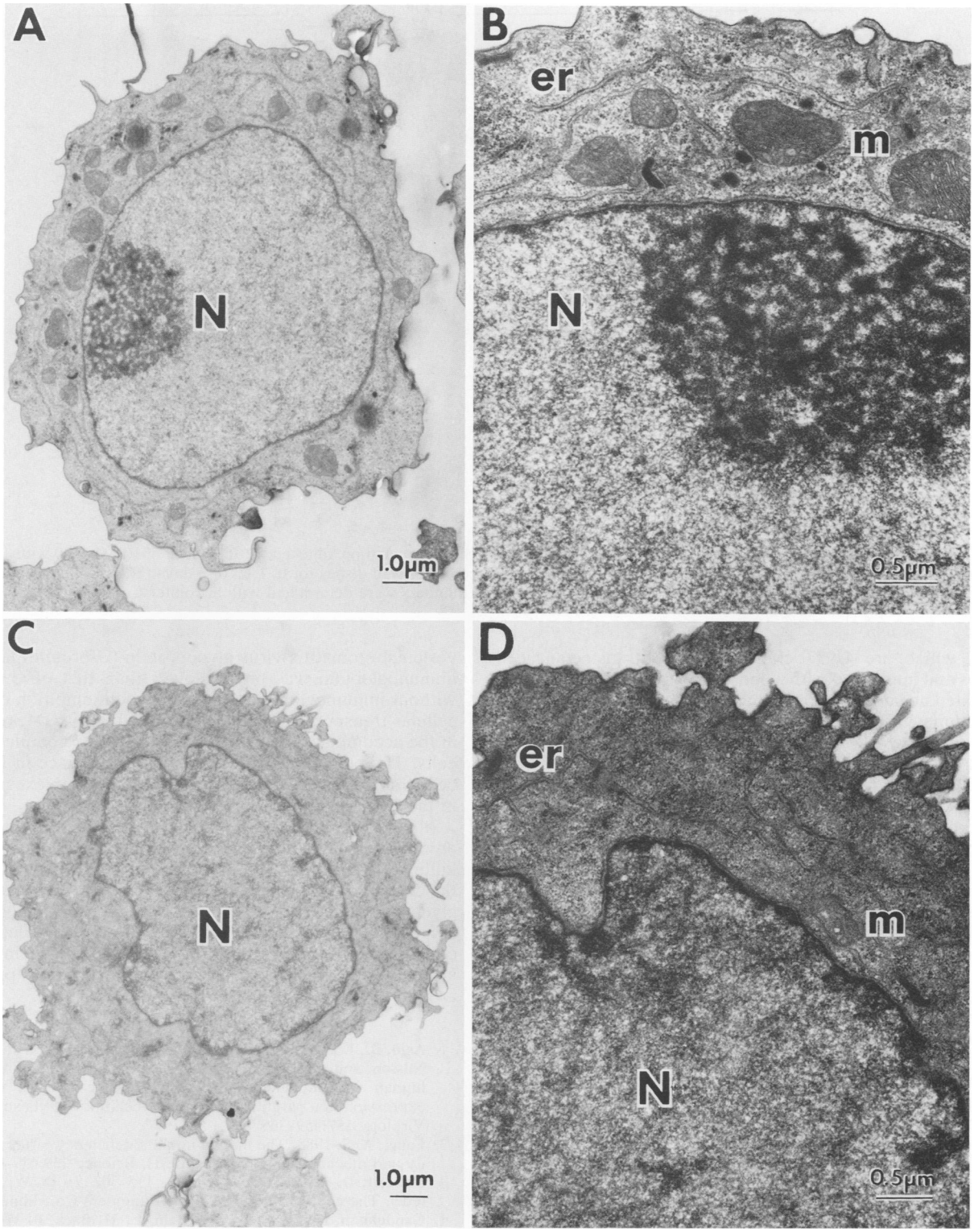

FIG. 8. Morphological analysis of UPME1 cells by TEM. UPME1 cells were treated with 10 μM $CdCl_2$, sampled at 0 h (A and B) and 8 h (C and D) after induction, and examined by TEM. Panels B and D are higher magnifications of panels A and C, respectively. Abbreviations are as described in the legend to Fig. 7.

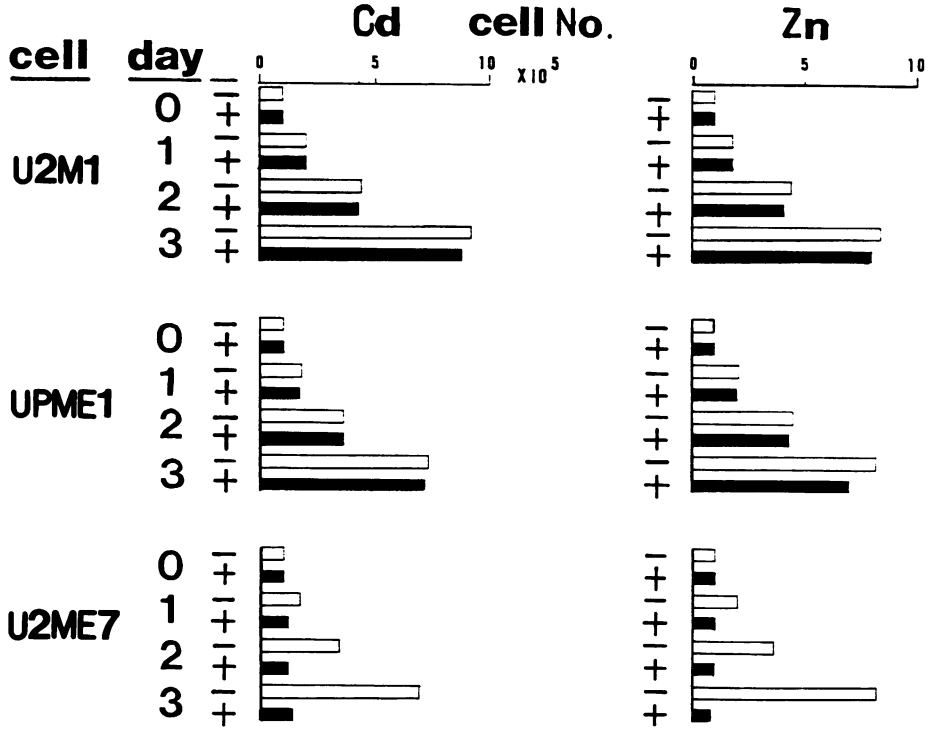

FIG. 9. Effect of expression of the *env* gene on growth of cells. Each cell population adjusted to 10^5 in 1 ml of RPMI 1640 with 10% fetal calf serum was treated with 10 μM $CdCl_2$ or 100 μM $ZnSO_4$ and cultured in 24-well dishes for 0, 1, 2, or 3 days (■). Cell cultures without metal ions were also prepared as controls (□). On each day the cell numbers were determined with a Coulter Counter.

cells, which are U937 clone 2-derived transfectant cell clones and integrated with a construct with a deletion of *env*, exhibited no such abnormal shape of nuclear envelopes after induction with cadmium by freeze-fracture or TEM analyses (data not shown), which excluded the possibility of any toxic effect of cadmium itself on such morphological and pathological events. In U2ME7 cells, cell growth was inhibited after the expression of envelope glycoprotein, whereas there was no such inhibition in U2M1 or UPME1 cells after induction.

The nuclear envelope consists of inner and outer nuclear membranes that enclose a perinuclear space. The two membranes are continuous with each other around circular pores through which the nucleoplasm appears to be in communication with the cytoplasm. The outer nuclear membrane is continuous with an endoplasmic reticulum (ER). Thus, functionally, small molecules and ions could diffuse across the nuclear membrane into the perinuclear space, and large molecules could pass through the pores directly into the cytoplasmic matrix or the nuclear matrix or vice versa (3). So the lesions of nuclear pores observed in our cell system may primarily inhibit the transportation between the nucleus and the cytoplasm of many molecules that are necessary for cell viability, resulting in the cytopathic effect.

The ER-Golgi complex system has been identified as a major site of cytoplasmic assembly of HIV in macrophage and monocyte cells (11). The CD4 molecule, a membrane-bound protein, thus is also transported to the plasma membrane by way of a Golgi complex after production in the ER (3). Therefore, in U2ME7 cells the gp160-CD4 complex may be formed in the ER system and then accumulate around the nuclear pores in the ER. Puddington et al. (13) reported that the rate of transport from ER to the Golgi complex of vesicular stomatitis virus glycoprotein (G protein) linked to immunoglobulin was much slower than that of G protein without immunoglobulin. In gp160, such inhibition of intracellular transport may happen by binding to CD4, resulting in the accumulation and condensation of the complex in the cells. The enlargement of the perinuclear space in U2ME7 cells found by TEM might be induced by complexes formed in the ER communicating with nuclear envelope.

It has been proposed that a high concentration of an envelope glycoprotein in cells is required for single-cell killing of HIV-infected $CD4^+$ T cells (17, 19). In such a case, the destabilization of the surface cell membrane by envelope glycoprotein accumulated in the cytoplasm is thought to be responsible for cell death. However, for the CD4-dependent cytopathic effect caused by envelope glycoprotein in our system, the primary site of the cytopathic effect is probably around the nuclear envelope.

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