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Role of the Cytoskeleton in Cell-to-Cell Transmission of Human Immunodeficiency Virus

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We previously observed that when human immunodeficiency virus (HIV)-infected T lymphocytes are added to epithelial cells, they adhere, polarize, and secrete virions unidirectionally onto the epithelium. Epithelial cells subsequently take up virus and become productively infected. We report here that colchicine treatment of T-lymphocyte suspensions induced lymphocyte polarization, redistribution of F-actin into a pseudopod, and secretion of HIV from the pseudopod. Immobilization of T lymphocytes on negatively charged plastic also caused redistribution of F-actin and unidirectional secretion of HIV onto the plastic. As neither colchicine nor adhesion caused an increase in HIV secretion, they apparently act by focusing secretion to the tip of the pseudopod. We speculate that adhesion-induced polar secretion of HIV, from activated mononuclear cells onto epithelia, is a cytoskeleton-mediated process which may be involved in HIV transmission in vivo.

The mechanism by which human immunodeficiency virus (HIV) is transmitted sexually across a genital or intestinal epithelium, or in utero across the placental trophoblast, is poorly understood. The requirement for lesions versus an intact epithelium has not been determined, and the role of cell-associated HIV versus cell-free HIV is unclear. However, indirect evidence from a number of in vivo (2, 12, 17–19) and in vitro studies (10, 22, 24, 32) suggests that an intact epithelium may be the target of HIV infection and that HIV-infected mononuclear cells present in blood (3, 11, 13, 16), semen (1, 15, 33, 34), and female genital secretions (28, 31) are the primary vectors in sexual transmission of HIV.

We previously developed an in vitro model that may imitate the way in which HIV is transmitted across an intact epithelium during sexual or transplacental transmission. To accomplish this, we grew epithelial monolayers of cell lines derived from the placenta, intestine, and cervix. Next, we added HIV-infected transformed T cells or monocytes. We observed that the HIV-infected, transformed mononuclear cells adhered to the epithelium and frequently became pear shaped (21). Within 30 min, we saw virions budding from the mononuclear cell onto the surface of the epithelium. Virus budding was seen exclusively on the region of the mononuclear cell where it was in contact with the epithelium. Within an hour after coculture, numerous virions were observed in the space between the adherent cells (4, 22, 24). Using a variety of techniques, we demonstrated that epithelial cells took up virions. We observed that the epithelial cells had subsequently incorporated HIV proviral DNA and were producing new virus (24, 27). We believe that this in vitro model may mimic the manner in which virus is transmitted in vivo.

We report here on a critical event in this model of cell-to-cell transmission of HIV, the mechanisms involved in directional secretion of virus from T lymphocytes onto the epithelium. By using agents which alter cytoskeletal elements, in association with an enzyme-linked immunosorbent assay (ELISA) to measure virus production and microscopic techniques, we have been able to elucidate some of the basic mechanisms involved in directional secretion of HIV. These include the role of microtubules and microfilaments, whether T cell-epithelial cell interaction stimulates increased secretion or only affects the direction of secretion, and whether the process requires specific molecular interactions. In addition to their relevance to sexual and transplacental transmission of HIV, the observations presented here are germane to basic characteristics of cells of the immune system and illustrate how retroviruses may subvert these properties.

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MATERIALS AND METHODS

Cell cultures. H9/HIV-1_{IIIB}, H9/HIV-1_{MN}, ACH-2/LAV, and CEM-SS T-lymphocyte cell lines were obtained from the AIDS Research and Reference Reagent Program, Division of AIDS, National Institute of Allergy and Infectious Disease, and MOLT4/HIV-1_{IIIB} T-lymphocytic cells were originally provided by J. Minowada. The human cervical epithelial cell line ME-180 was purchased from the American Type Culture Collection (Rockville, Md.). All cells were maintained in RPMI 1640 medium with 10% heat-inactivated fetal bovine serum (FBS).

Reagents. Reagents were purchased from Sigma (St. Louis, Mo.). Ten millimolar stock solutions of colchicine (C-9754), lumicolchicine (L-0635), nocodazole (M-1404), vinblastine (V-1377), taxol (T-7402), and 4.2 mM cytochalasin B (C-6762) were made up in dimethyl sulfoxide (DMSO; D-2650; Sigma) and stored at 4°C.

Electron microscopy. Methods for electron microscopy have been described previously (24).

Time-lapse cinematography. For time-lapse cinematography, MOLT4/HIV-1_{IIIB} lymphocytic cells (3×10^5/ml) were added to 25-cm^2 T flasks (Corning Glass Works, Corning, N.Y.) in the presence of one of the following: 20 μM colchicine; 20 μM cytochalasin B; 15-min pretreatment with 20 μM taxol followed by the addition of 20 μM colchicine; or 0.2% DMSO solvent control. The cap was screwed on to the flask, and the culture was transferred immediately to a Nikon

inverted microscope equipped with a 37°C chamber and Wild-Boulex 16-mm time-lapse recording system as previously described (21). Cells were photographed over a 3-h period.

Cytochemistry. A stock solution (60 μg/ml) of fluorescein isothiocyanate (FITC)-conjugated phalloidin (Sigma) was made up in methanol and diluted in phosphate-buffered saline (PBS) for use as described by (29). MOLT4/HIV-1_{IIIB} cells, which had been pretreated for 1 h with 0.2% DMSO or 20 μM colchicine, were fixed in 2% paraformaldehyde for 30 min at 4°C. Cells were washed three times in PBS–0.1% bovine serum albumin (BSA) and permeabilized in 95% ethanol for 20 min at room temperature. Cells were washed three times in PBS–0.1% BSA and incubated with FITC-phalloidin (0.6 μg/ml) for 30 min in the dark at room temperature. Cells were then washed twice in PBS and resuspended (5×10^6/ml) in Vectashield antifade (Vector Laboratories, Burlingame, Calif.). One drop of this suspension on a glass slide was covered with a no. 1 glass coverslip and viewed in a Zeiss fluorescence microscope.

For cytochemical staining of plastic adherent MOLT-4/HIV-1_{IIIB} lymphocytes, a twofold serial titration of lymphocytes (2×10^6 to 6×10^4/ml) was incubated on eight-well plastic chamber slides (Lab-tek; Nunc Inc., Naperville, Ill.) for 40 min in serum-free RPMI at 37°C in 5% CO_2. Cells were then fixed in 2% paraformaldehyde for 10 min at room temperature, washed in PBS–0.1% BSA, permeabilized in 95% ethanol, and prepared for FITC-phalloidin staining as described above.

p24 measurement of colchicine-treated lymphocytes. MOLT4/HIV-1_{IIIB} cells were washed five times in serum-free medium to remove residual p24 from the culture medium. Lymphocytes (10^4/ml) were resuspended in either 0.2% DMSO (control) or 20 μM colchicine, in 10% medium, and cultured in 24-well plates (Falcon; Becton Dickinson and Co., Lincoln Park, N.J.) for 6 h. At timed intervals, medium was removed from each well and spun in a Beckman microcentrifuge at 1,500 rpm for 2 min to pellet the cells. Supernatants were retained, stored at −70°C, and assayed the following day for p24 HIV-1 core antigen by ELISA (Coulter, Hialeah, Fla.) as instructed by the manufacturer.

Lymphocyte adhesion to tissue culture-treated plastic. MOLT4/HIV-1_{IIIB} cells were washed two times in serum-free medium, resuspended (10^5/ml for scanning electron microscopy [SEM] and 10^6/ml for transmission electron microscopy [TEM]) in serum-free medium, and incubated on 24-well plates for 40 min. Wells were washed two times in 0.2 M phosphate buffer (pH 7.3), fixed in 1.25% glutaraldehyde, and observed in the SEM and TEM.

p24 measurement of plastic-adherent and epithelial cell-adherent lymphocytes. MOLT4/HIV-1_{IIIB} cells were washed five times in serum-free medium, resuspended (10^4/ml) in either serum-free medium or 10% medium, and cultured in 24-well plates as described above. Lymphocytes were also added (in 10% medium) to confluent monolayers of ME-180 epithelial cells seeded on 24-well plates. At timed intervals, medium was removed from each well and assayed for p24 core antigen as described above.

RESULTS

Cytoskeleton and polar secretion. We have previously proposed that transmission of HIV may entail a cell-to-cell entry mechanism involving (i) HIV-infected cells of the immune system in body fluids and (ii) intact CD4-negative epithelial cells at the site of entry (4, 24). This process is initiated by adherence of HIV-infected cells to epithelia. Adherence results in secretion of virus into a space between the infected mononuclear cell and the epithelium and uptake of virions by the epithelial cells (21). We have also demonstrated that the epithelial cell becomes productively infected and new virus is secreted (22, 27).

In the course of investigating the directional secretion of HIV from HIV-infected transformed T cells, we noted that the T cells frequently became pear shaped soon after they adhered to the epithelium (21, 24). As cytoskeletal elements are known to mediate secretion and changes in cell shape in other systems, we carried out the following experiments to determine the role of microtubules and microfilaments in the formation of pear-shaped cells and unidirectional secretion of HIV.

(i) Time-lapse cinematography. To determine the role of the cytoskeleton in adhesion-induced lymphocyte shape change, MOLT4/HIV-1_{IIIB} cells were treated, in suspension, with colchicine (20 μM). Time lapse cinematography was used to monitor changes in shape with time. As soon as they settled, about 5 min, we observed that most of the cells were irregular in shape and usually contained one or more small lobate processes. Frequently they were pear shaped. By 15 min, processes were seen in nearly all of the cells (Fig. 1b). The formation of these processes was dynamic; that is, over a period of 3 h, processes could be seen to extend and retract, and new processes were seen to form on a different region of the cell. Control (DMSO-treated) cells were generally spherical, although many of the control cells displayed long filamentous processes (Fig. 1a). The colchicine-induced shape changes were due to the specific effect of colchicine on microtubules; lumicolchicine had no effect, whereas nocodazole and vinblastine, two drugs which inhibit tubulin polymerization, had the same effect as colchicine on cell shape, inducing aberrant shape changes with small lobar processes. The microtubule-stabilizing agent taxol prevented polarization of cells in suspension, and cytochalasin B, which prevents actin polymerization, caused cells to round up.

(ii) Cytochemistry. It has previously been shown that polymorphonuclear leukocytes (PMNs) change shape from round to polar, increase F-actin content, and shift F-actin distribution from a diffuse cytoplasmic distribution to a focal distribution in response to chemotactic peptides (14). F-actin also appears to redistribute into the area of attachment between cytotoxic T lymphocyte-target cell conjugates (26) and natural killer cell-target cell conjugates (5). To determine whether colchicine induced a focal distribution of F-actin in HIV-infected T cells, we stained colchicine-treated T cells with FITC-phalloidin. In control (DMSO-treated) cells, staining was observed mainly around the periphery of the cell and diffusely in the cytoplasm. However, in colchicine-treated cells, staining was localized in the pseudopod. Thus, colchicine induced a focal distribution similar to that which has been reported to occur in PMNs and lymphocytes (Fig. 2).

(iii) Electron microscopy. Electron microscopy was used to determine the relationship between colchicine-induced pseudopod formation and viral secretion. MOLT4/HIV-1_{IIIB} cells are a high-virus-producing cell line. In control cells, viral budding is only occasionally observed in the TEM. However, mature virions are frequently seen associated with the plasmalemma, as virions remain associated with the cell membrane at the site of budding for some time after they have formed. Rather than occurring uniformly on the cell surface, virus formation takes place in one or several sites on the surface of the cell (23).

When colchicine-treated MOLT4/HIV-1_{IIIB} cells were observed in the TEM, we observed that budding occurred almost exclusively on the tip of the pseudopodium and that virions

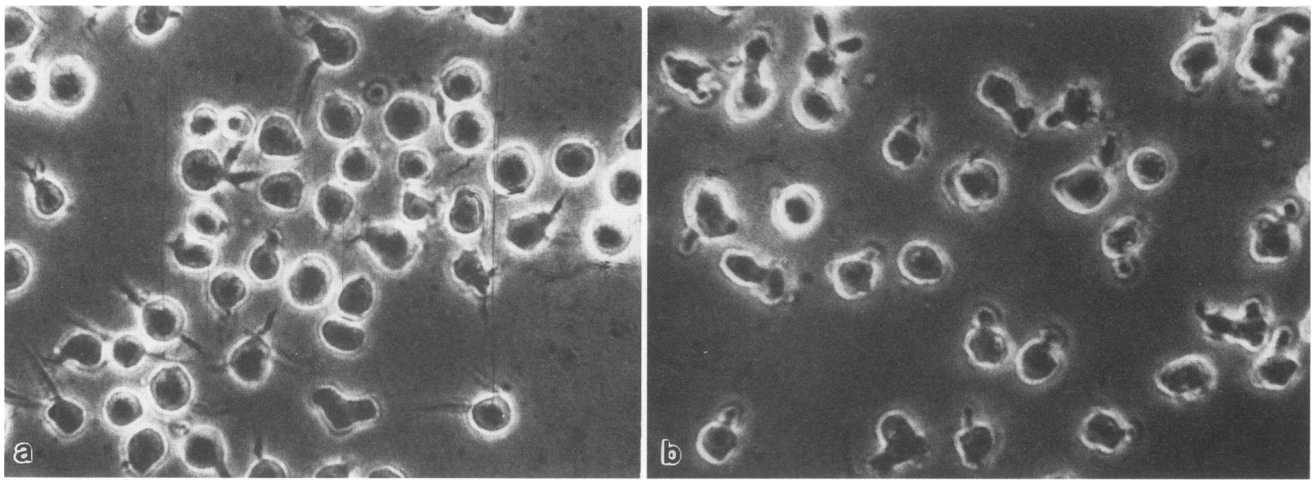

FIG. 1. Phase-contrast micrographs of untreated (a) and colchicine-treated (b) MOLT4/HIV-1_{IIIB} cells 15 min after the addition of the drug. Control cells are spherical and sometimes have a long filamentous process. Colchicine-treated cells display irregular shapes and frequently have a lobate pseudopod. Magnification, ×450.

were associated with this area of the cell (Fig. 3). H9/HIV-1_{IIIB} and H9/HIV-1_{MN} cells were also found to polarize and secrete virions from their newly formed pseudopod extensions.

(iv)Effect of colchicine on virus production. The findings discussed above seem to indicate that colchicine rapidly causes MOLT4/HIV-1_{IIIB} cells to form pseudopods through a mechanism involving movement of F-actin to the tip of the pseudopod. Viral budding is also seen at the tip of the pseudopod. However, since these chronically infected T cells are already producing virus, it is not clear whether colchicine is inducing an increase in virus production or simply causing virus to be secreted exclusively from the tip of the pseudopod, without increasing the rate of HIV secretion. To answer this question, we used p24 ELISA to monitor viral production over a 6-h treatment period. This experiment was repeated several times under slightly different conditions. The results were consistent. Colchicine had no effect on HIV production (Fig. 4).

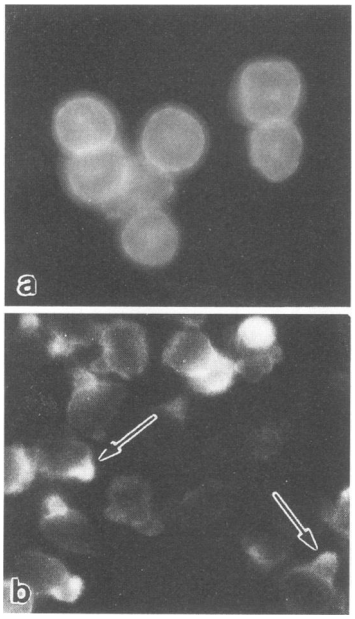

FIG. 2. Distribution of F-actin as shown by FITC-phalloidin labeling. (a) Control; F-actin is distributed around the periphery of the cell and homogeneously in the cytoplasm. (b) Colchicine; label at the tip of the pseudopod (arrows). Magnification, ×450.

Effect of adherence on HIV secretion. (i) Redistribution of F-actin. Several years ago, when we first observed polar secretion of HIV onto the surface of epithelia, we felt that directional secretion was likely to involve cytokine-mediated cross-talk between the adherent cells. However, when we recently saw that simply preventing polymerization of microtubules could mimic the phenomenon, we asked whether adhesion to a surface other than an epithelium could also induce polar secretion. To study nonspecific adhesion, we plated HIV-infected T cells in serum-free medium. When T cells are plated in medium containing serum, they do not adhere to the plastic because the serum rapidly binds to and coats the negatively charged plastic of the culture well. However, when plated in serum-free medium, the MOLT4/HIV-1_{IIIB} cells adhere. Observation of FITC-phalloidin-stained adherent HIV-infected T cells, 40 min after they were added to plastic, revealed that F-actin was primarily localized at the base of the cell. Fluorescent staining was seen as a ring, as would be expected if distribution was associated with the plasma membrane of a pseudopod (Fig. 5).

(ii) Electron microscopy. Viewed in the SEM 40 min after plating, the plastic-adherent cells often displayed shapes similar to those of cells that had been treated with colchicine and to those of cells which we had previously observed adhering to epithelia (21, 24). T cells were also apparently induced to form microvilli by adhering to plastic, as prominent microvilli were observed at the base of the cells (Fig. 6). The most interesting aspect of adhesion to plastic was polar secretion of HIV. This phenomenon was striking when cells were viewed with the SEM. In many cells, hundreds of virions were seen in the area immediately adjacent to the plastic, among microvilli, and on the plastic adjacent to the cell. However, virions were virtually absent from the rest of the cell surface (Fig. 6). To confirm that the small spherical structures which we had observed at the base of the adherent HIV-infected T cells were virions, we prepared some of the cultures for the TEM. In sections cut parallel to the plastic through the portion of the cell which was

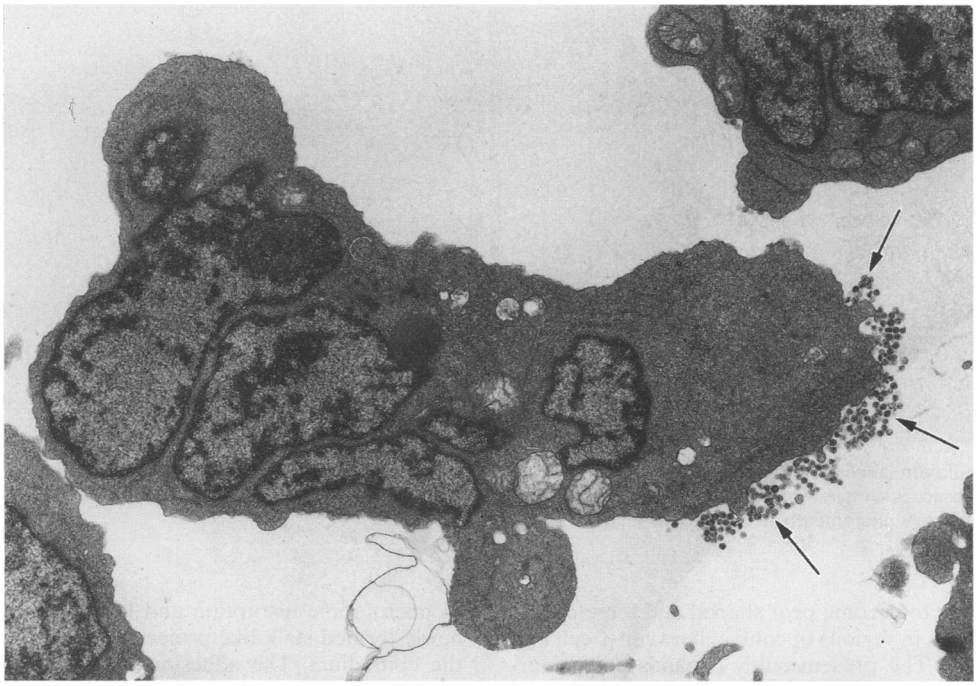

FIG. 3. TEM of a MOLT4/HIV-1_{IIIB} cell 40 min after the addition of colchicine. Virions are observed exclusively on the tip of the pseudopod (arrows). Magnification, ×8,000.

adherent to the plastic, we observed numerous cellular processes, and among them we found hundreds of HIV virions (Fig. 7). In contrast, in sections which transected the cell further away from the base of the cell, virions were either absent or very few in number.

(iii) Effect of adhesion on virus production. We used p24 ELISA to determine whether adherence stimulated production of HIV (Fig. 8). Comparisons were made between (i) MOLT4/HIV-1_{IIIB} cells in RPMI with 10% FBS on plastic, (ii) MOLT4/HIV-1_{IIIB} cells in RPMI without serum on plastic, and (iii) MOLT4/HIV-1_{IIIB} cells in RPMI with 10% FBS on confluent monolayers of ME-180 cells (epithelial cell line derived from the human cervix). This experiment was repeated several times. We consistently observed that the highest production was when cells were plated onto plastic in the presence of medium with serum, conditions in which they do not adhere to the plastic. There was somewhat less virus in the medium when cells were plated onto the ME-180 epithelium and even less when cells in serum-free medium were plated onto plastic. When the experiment was done with the latent T-cell clone ACH-2/LAV (a low-virus-secreting cell line), the results were similar, suggesting that this cell line was not stimulated to secrete more HIV following adhesion. We were not surprised to find that less virus was in the medium when we cocultured HIV-infected cells with epithelial cells, as we have previously observed that numerous virions are taken up by the ME-180 epithelial cells (27). The difference between production in serum-free versus medium containing FBS may be due, in part, to HIV adsorption to the plastic and the fact that RPMI without serum is not a sufficient medium for MOLT4/HIV-1_{IIIB} cells. In any event, since the least amount of virus was produced under conditions which promoted adherence, these results indicate that adherence does not stimulate virus production.

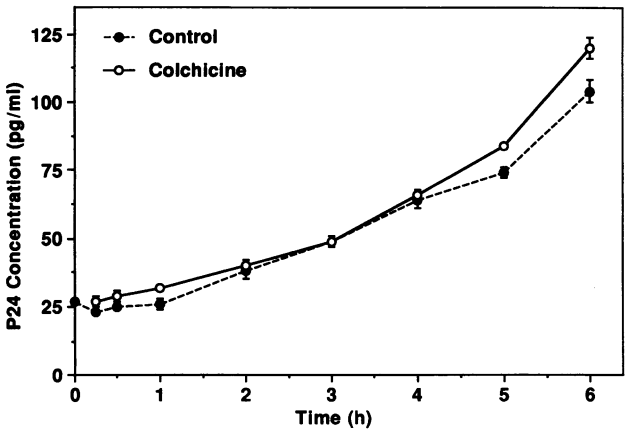

FIG. 4. ELISA of p24 concentration versus time. The concentration of p24 increases with time, and there is no significant difference in p24 values between colchicine-treated lymphocytic T cells (○) and DMSO-treated control cells (●). Thus, colchicine does not stimulate an increase in HIV secretion. Data points represent the mean of two wells (± standard deviation); the experiment was repeated four times with similar results.

DISCUSSION

The experiments described above are based on an in vitro model of sexual transmission of HIV, which may be appropriate to the situation in vivo during sexual and transplacental transmission of HIV (4, 21, 22, 24, 27). The model consists of chronically HIV-infected T cells or monocytes coincubated with a confluent epithelial monolayer. We have shown that T cell-to-epithelial cell adhesion is a prerequisite for cell-mediated HIV infection of the epithelium. When the lymphocytes

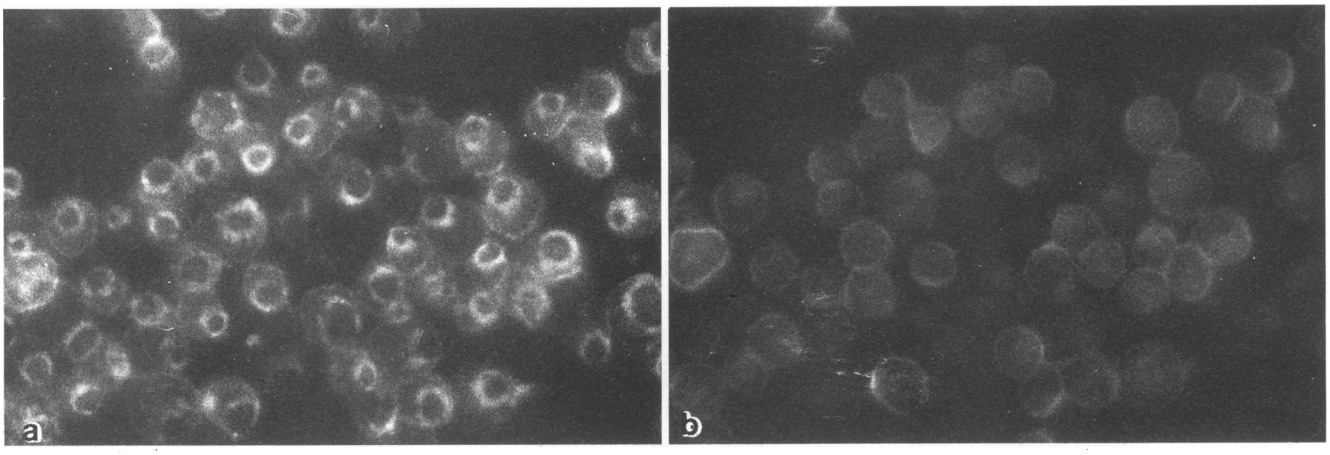

FIG. 5. FITC-phalloidin labeling of adherent HIV-infected T lymphocytes 40 min after addition to plastic. When the focal plane is at the level of the plastic, fluorescence is seen as a small bright ring at the base of the cell (a). Diffuse, faint staining is observed when the focal plane is through the center of the cell a few micrometers above the plastic (b). Magnification, ×450.

adhere, they polarize to become pear shaped and secrete HIV toward the epithelium in regions of contact between T cell and epithelial cells only. The present study expands our understanding of adhesion-mediated lymphocyte polarization and directional HIV secretion onto an epithelium.

When HIV-infected lymphocytes were treated in suspension with colchicine to prevent microtubule polymerization, they became pear shaped and resembled the morphology that we observe when HIV-infected lymphocytes adhere to epithelia. Cytochemical staining of the colchicine-treated lymphocytes revealed F-actin in the newly formed pseudopod extension.

By analogy, these results suggest that the shape changes induced by lymphocyte-to-epithelial cell adhesion could be due to microtubule disruption and F-actin redistribution into the newly formed stalk-like projection which tethers the T cell to the epithelium. The adhesion-induced changes in cell shape that we observe are not unique to our in vitro system. Dustin et al. (9) observed that a B-lymphoblastoid cell line will attach to a lipid bilayer, containing LFA-1, through a pseudopod stalk-like structure, and they also found that ICAM-1 accumulated at the end of the stalk in contact with the bilayer containing LFA-1. Localization of ICAM-1 to the pseudopod of HSB2, a T-lymphocyte cell line which forms homotypic aggregates in suspension, has also been described by Dougherty et al. (8). Furthermore, when human peripheral blood lymphocytes are activated, they polarize by expressing a pseu-

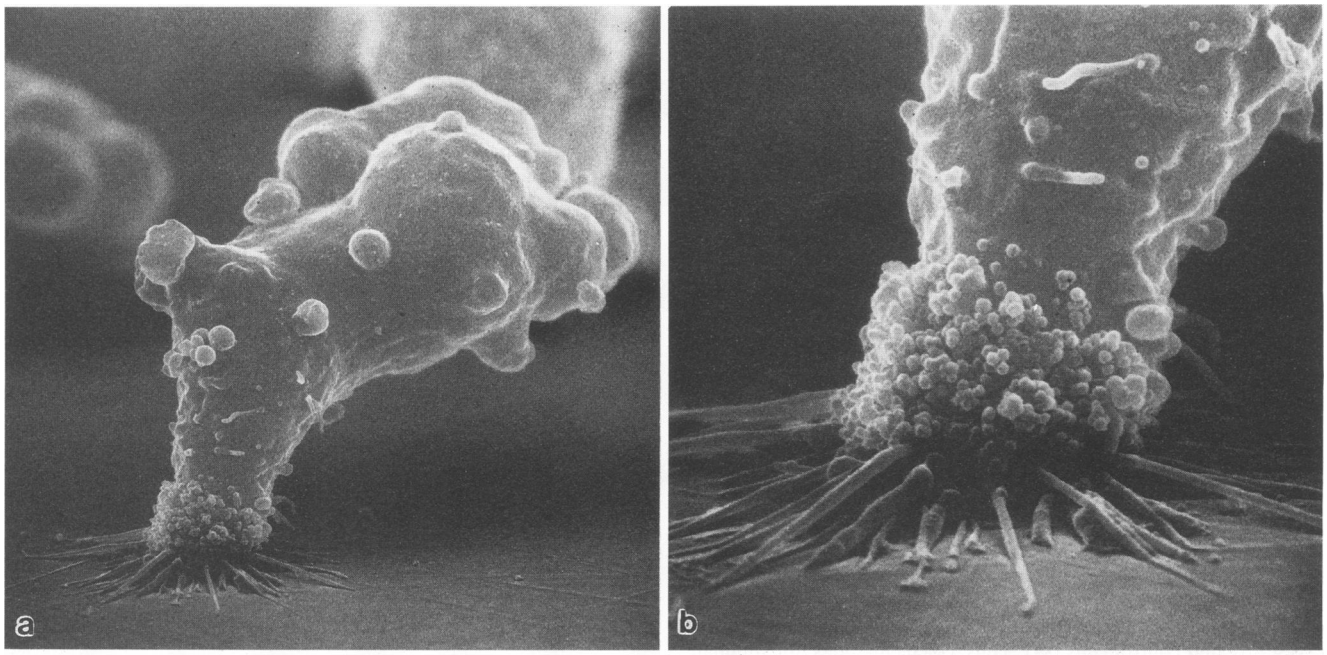

FIG. 6. (a) SEM of a MOLT4/HIV-1_{IIIB} cell 40 min after being plated onto plastic in serum-free medium. The cell has an irregular shape, similar to cells treated with colchicine. (b) Higher magnification of the same cell. Microvilli emanate from the base of the cell. Numerous virions are seen on the base of the cell. Magnifications: (a) ×6,000; (b) ×17,000.

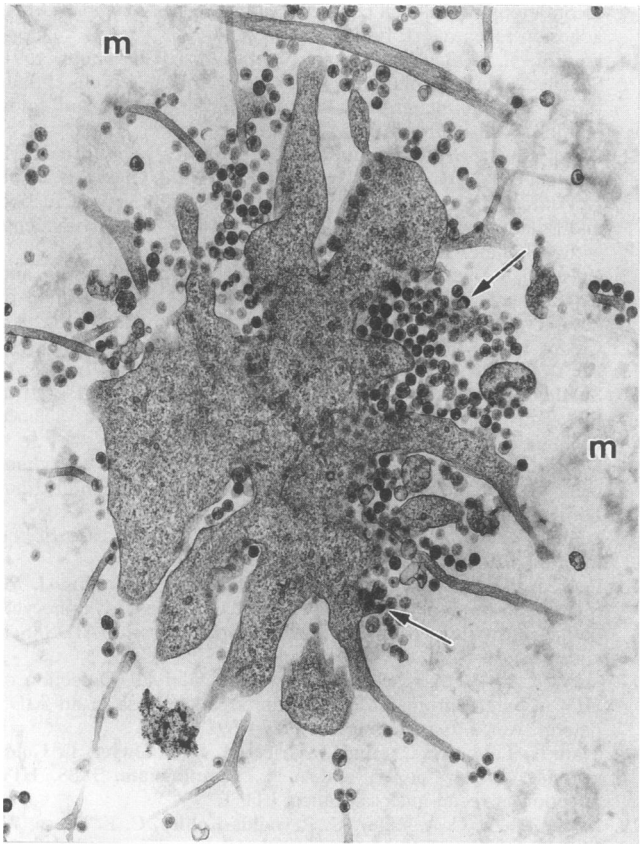

FIG. 7. TEM through the base of a MOLT4/HIV-1_{IIIB} cell 40 min after being plated onto plastic in serum-free medium. The matrix material (m) indicates that this section has transected the cell immediately above the plastic. Numerous mature and budding virions (arrows) are observed in association with the plasmalemma. Magnification: ×18,000.

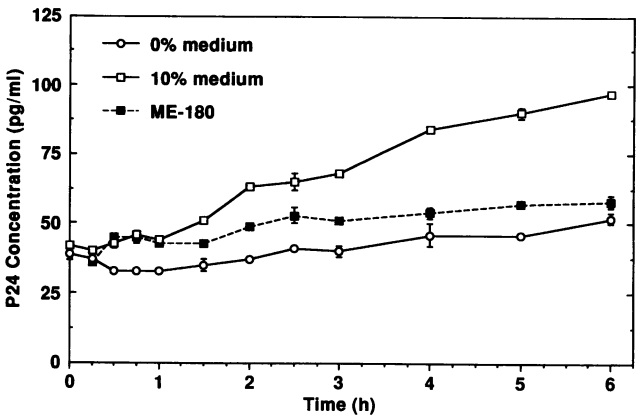

FIG. 8. p24 measurement of plastic-adherent (○) and epithelial cell-adherent (■) lymphocytic cells versus nonadherent conditions (□). The concentration of p24 increased with time under these conditions, but the highest production was detected when cells were cultured on plastic in the presence of medium containing serum (i.e., under nonadherent conditions). In the presence of epithelial cells (ME-180), there was less p24, probably because of adsorption and uptake of HIV by the epithelium. In serum-free medium, lymphocytes adhered to plastic and there was somewhat less p24 compared with lymphocytes cultured in the presence of the epithelial cells. This may be due, in part, to HIV adsorption to plastic and the requirement of FBS to maintain cells. Data points represent the mean of two wells (± standard deviation); the experiment was repeated four times with very similar results.

dopod and become motile (30), both of which are characteristics of activated lymphocytes (20). Cytotoxic lymphocytes also exhibit motility and extend a pseudopod against target cells during target cell lysis (25, 26), as do natural killer lymphocytes (5).

Cell shape changes can also be demonstrated in suspension using *N*-formyl-L-methionyl-L-leucyl-L-phenylalanine-activated neutrophils (PMNs). As discussed above, activated PMNs change from a round to a polar morphology. The shape change is associated with a redistribution of cytoskeletal microfilaments, since F-actin shifts from a diffuse cytoplasmic distribution to a focal distribution in the pseudopod (14). Cytotoxic T lymphocytes and natural killer lymphocytes also redistribute microfilaments into the region of a cell in contact with a target cell (5, 26).

The relationship of F-actin distribution to development of polar shape in PMNs has been well studied by Coates et al. (6), who show that after stimulation, F-actin polymerizes first at the rim of the PMN, which is followed by asymmetry in F-actin distribution and change to polar shape. Asymmetric F-actin distribution precedes polar shape, and F-actin localizes in the pseudopod. Thus, PMNs activated with chemotactic peptides undergo polarization and an increase in F-actin content. It is also possible to polarize basal PMNs without activating them. This is achieved by using the microtubule-disrupting agent nocodazole. Under these conditions, the amount of F-actin does not increase but shifts position from a diffuse distribution in round PMNs to a focal distribution in the extended pseudopod of the polarized PMN (7). Conry et al. (7) suggest that microtubules interact with microfilaments to "maintain a symmetric F-actin distribution, and disassembly of microtubules allows a redistribution of F-actin in PMN polarization, without causing a net change in F-actin content."

Interestingly, we found that colchicine induced not only a pear-shaped morphology but also an associated unidirectional secretion of HIV from the pseudopod extension of the colchicine-stimulated cells. Colchicine induces a directional secretion of HIV without increasing the amount of secreted virus, compared with the control chronically HIV infected lymphocytes. This finding suggests that the observed polarization in our in vitro model may promote secretion of HIV into the region of contact between the lymphocyte and the epithelium. Furthermore, it appears that these high-secreting, chronically HIV infected cell lines, which secrete HIV at a certain rate, adhere to a target cell and secrete HIV at the same rate but unidirectionally into the region of contact between the opposing lymphocyte and epithelial cell. This result also suggests that the epithelium does not stimulate the lymphocytes to secrete HIV but tethers the lymphocytes.

To investigate this phenomenon further, HIV-infected lymphocytes were immobilized on plastic. This hydrophilic surface with a net negative charge induced T cells to polarize into pear shapes, localize F-actin, and secrete HIV unidirectionally onto the plastic. The data suggest that the shape changes induced by colchicine and those induced by binding to a plastic substratum are mediated by the same mechanism, namely, redistribution of F-actin to the pseudopod. Furthermore, colchicine and tissue culture-treated plastic appear to mimic the effect of an intact epithelium i.e., inducing a shape change and directional HIV secretion, either by disrupting microtubules directly or by immobilizing lymphocytes via charge interactions which may

serve to cross-link cell surface molecules. Neither colchicine nor adhesion to a plastic surface stimulated an increase in HIV secretion, and when lymphocytes were incubated on a confluent epithelium, there was no detectable increase in HIV secretion. We have previously reported an increase in HIV production, 30 min following adhesion to an epithelial monolayer, compared with lymphocytes cultured in the absence of an epithelium (22). However, in the experiments reported here, we have found that adhesion does not stimulate an increase in HIV secretion. Since we have repeated these experiments several times with similar results, we suspect that our previous report was in error.

If our findings are relevant to the situation in vivo, they suggest that chronically HIV infected mononuclear cells, present in genital tract secretions or blood, could adhere to epithelia which line the genital tract or placenta and within minutes polarize and secrete HIV virions onto the epithelium. The epithelium serves to immobilize the lymphocytes and triggers a morphological polarization and unidirectional secretion of HIV but the mononucleocytes do not require cell activation signalling.

We caution that the experiments described here have been conducted with transformed cell lines. Studies carried out with normal lymphocytes or macrophages as well as normal epithelial cells are needed to determine the relevance of our findings to sexual and transplacental transmission of HIV.

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