Arch Virol (1996) 141: 1177–1189

Archives of Virology

© Springer-Verlag 1996
Printed in Austria

Cell-mediated infection of cervix derived epithelial cells with primary isolates of human immunodeficiency virus

X. Tan and **D. M. Phillips**

The Population Council, New York, U.S.A.

Accepted March 5, 1996

Summary. We have previously demonstrated that HIV-infected transformed T-cells or monocytes adhere to monolayers of CD4-negative epithelial cells. Adhesion is soon followed by budding of HIV from infected mononuclear cells onto the surface of epithelial cells. Epithelial cells subsequently take up virus and become productively infected. Based on these findings, we proposed that sexual transmission of HIV may involve cell-mediated infection of intact mucosal epithelia of the urogenital tract. However, it has become increasingly clear that primary cells and HIV strains isolated from patients are more appropriate models for HIV infection than established cell lines and lab strains of virus. In the studies described here, we infected cervix-derived epithelial monolayers with primary monocytes infected with patient isolates of non-syncytial inducing (NSI) macrophage-tropic strains of HIV. Under the culture conditions employed, HIV-infected primary monocytes do not remain adherent to the apical surface of the epithelium, as did HIV-infected transformed cells. Instead, following adherence, the primary cells migrate between epithelial cells. Virus is secreted from a pseudopod as HIV-infected primary monocytes pass between cells of the epithelium. Productive infection of the epithelium was detected by p24 ELISA and PCR Southern blot analysis. Infection can be blocked by sera from HIV-seropositive individuals or by certain sulfated polysaccharides. These findings support the supposition that transmission of HIV may occur via cell-mediated infection of intact epithelia. The observations also hint at the possibility that HIV-infected monocyte/macrophages in semen or cervical-vaginal secretions could cross intact epithelia by passing between epithelial cells. Blocking studies suggest that it may be possible to inhibit sexual transmission of HIV either by antibodies in genital tract secretions or by a topical formulation containing certain sulfated polysaccharides.

Introduction

The importance of understanding the mechanisms of sexual transmission of HIV is obvious; 90% of all adult HIV infections were obtained through sexual contact

[1]. Unfortunately, many of the fundamental aspects of sexual transmission of HIV are poorly understood. It is not clear how frequently infection occurs through lesions in the genital tract epithelium and how often infection takes place via an intact epithelium. Other weaknesses in our understanding are that the site(s) of infection (e.g., vagina, cervix, urethra, vulva, foreskin) has not been determined, and the target cell types (e.g., stratified squamous epithelium, simple columnar epithelium, Langerhans cells, M cells) are not known. In addition, there is controversy over whether infection is mediated by cell-free virus or HIV-carrying mononuclear cells. If transmission is cell mediated, it is not clear whether the cellular vectors are monocyte/macrophages or lymphocytes. In addition, it is not apparent whether or not certain strains of HIV are more easily transmitted than others.

We previously developed in an vitro model to study the way in which HIV might be transmitted during coitus. To accomplish this, we grew epithelial monolayers of cell lines derived from the human cervix and added HIV-infected transformed T-cells. HIV-infected T-cells were seen to adhere to the epithelial cells. Adherence resulted in the budding of virus from the T-cell onto the surface of the epithelium, and within an hour after co-culture, numerous virions were observed in the space between the adherent cells [17, 18, 20, 23]. The epithelial cells subsequently incorporated HIV proviral DNA and produced new virus [23]. Virus production can be demonstrated by ELISA [20, 21] and in the case of the ME180 cell line used in the present study, so much virus is produced that HIV secretion can be visualized in the electron microscope [22, 23]. Based on these observations, this in vitro model may mimic the manner in which virus is sexually transmitted in vivo [18, 22, 23].

However, our previous in vitro model could be improved. Mononuclear cell lines differ in many ways from normal mononuclear cells. In addition, the cellular vectors of HIV are likely to be monocyte/macrophages rather than lymphocytes, since viruses isolated from patients infected through sexual contact and in the acute phase of infection are primarily macrophage tropic [25]. This makes sense, theoretically, as monocytes are more abundant than lymphocytes in semen [24], and are more long lived than lymphocytes [11]. However, since cell HIV-1 variants replicate in primary lymphocytes, it is also feasible that macrophage tropic variants could be transmitted by T helper cells. Selective pressure may be exerted in the mucosal tissue allowing only the macrophage tropic variants to replicate.

Considerable evidence has shown different properties in lab strains as compared to patient isolates of HIV. Two of the most significant differences are: 1) primary isolates generally do not infect T-cell lines [25] and 2) human antisera to laboratory strains of HIV do not block in vitro infection of primary isolates [14].

We report here on an in vitro system which employs primary activated monocytes which were infected with macrophage-tropic virus strains isolated from patients. Although HIV-infected, activated primary monocytes readily infect cervix-derived cells, the mechanism of interaction between the monocyte

and the epithelium is different from that between HIV-infected lymphoma cells and epithelia. This suggests that a different mechanism of infection, than we had proposed previously, may operate during coitus. Blocking experiments were also performed. The findings of these studies are very encouraging as they point to strategies which may be able to inhibit sexual transmission of HIV.

Materials and methods

ME180 cells

The human cervical epithelial cell line, ME180, was purchased from the American Type Culture Collection (Rockville, MD). MOLT-4, HeLa and HT-6C cell lines were obtained from the AIDS Research and Reference Reagent Program, Division of AIDS, NIAID (ARRRP). All cells were maintained in RPMI-1640 medium with 10% heat-inactivated fetal bovine serum. Medium for HT-6C cells was supplemented with 10 μg/ml neomycin (GIBCO, Grand Island, NY)

Preparation of HIV-1 infected monocytes

Ten ml of semipurified leukocytes from normal, HIV-seronegative donors (obtained from the Greater New York Blood Center, NY, NY) were diluted in 30 ml RPMI-1640 over 15 ml of Histopaque-1077 solution (Sigma, St Louis, MO) and centrifuged for 30 min at 400 **g**. The mononuclear cell fraction was seeded at 10^7 cells/ml in RPMI-1640 containing 10% FBS and 5 μg/ml PHA (GIBCO, NY). After 48 h the PBMC were infected with either primary isolates of HIV-1 strain P1–2 or O/S (gifts from Drs. Ruth Connor and David Ho), or monocyte-tropic strain AdaM (ARRRP). Seven days later, infected PBMC were mixed 1:5 with freshly isolated non-infected PBMC. Cells were seeded at 10^7/ml. After seven days, non-adherent cells were removed by washing with PBS. Virus production was monitored by p24 ELISA of the medium after 24 h of culture. The cells were used if the p24 production was greater than 50 ng/10^6 cells. It was previously determined that the cultures reached peak HIV-production at about 7 days post infection. HIV production remained at about this level for a few days. For co-culture, monocytes were removed from the plastic at 7 days post infection by treatment with 0.02% EDTA in PBS at RT. When cells were no longer adherent (usually about 5 min) they were removed, washed in medium and treated with 200 μg/ml mitomycin C (Sigma, St Louis, MO) in culture medium for 1 h. The monocytes were then washed in culture medium and added to epithelial cultures as described below.

Immunocytochemistry

HIV infected monocytes or PBMCs, isolated as described above, were permeabilized with 0.2% Tween 20 for 10 min, washed and incubated for 1 h at RT with either monoclonal antibody to CD68 (Accurate Chemical, Westbury, NY). Non-permeabilized cells were stained with monoclonal antibody to CD14 (Sera-lab Sussex, England). After washing in PBS, cells were stained with FITC labeled anti-mouse IgG at a dilution of 1:100 (Boehringer Mannheim, Indianapolis, IN).

Mitomycin C treatment

To determine the concentration of mitomycin C which would kill PHA stimulated HIV-infected monocytes, wells were incubated with various concentrations of mitomycin C for 1 h.

Following treatment, activated monocytes were washed and cultured for 24 or 48 h. Viability was accessed by the MTT assay [15].

Monocyte-mediated infection of epithelial cells

ME180 cells were seeded in six-well tissue culture plates (Becton Dickinson, Bedford, NJ) at a density of 2×10^5 cells/well and cultured for 24 h at 37 °C in 5% CO_2 incubator. 5×10^5 HIV-1 infected monocytes (prepared as described above) were added to each well. After six hours, the culture was washed six times with culture medium. The medium was changed every 24 h for 5 days. On the 6th day the medium was assayed for HIV using a p24 antigen ELISA kit (Coulter, Hialeah, FL).

Blocking assays

The following reagents were employed in blocking studies. Human immunoglobulin was prepared from the plasma of healthy HIV seropositive donors (APPPR). This sera was 98% IgG and had high neutralizing ability. Polyclonal rabbit anti-gp120 antibody was obtained from American Bio-Technologies, (Cambridge, MA) and dextran sulfate and heparin from Sigma (St Louis, MO). Reagents were added to culture medium and pre-incubated with ME180 cells for 30 min before co-culture with HIV-1 infected monocytes. Incubation and ELISA assays were performed as described above.

RT-PCR and southern blot hybridization for CD4 expression

A single-step method was used to extract total RNA [7]. Reverse transcription of RNA and PCR amplification of cDNA was carried out using GeneAmp RNA Kit as described by the manufacturer (Perkin-Elmer, Norwalk, CT). One μg RNA was mixed with 1 mM dNTP, 1 U/μl RNase inhibitor, 2.5 U/μl reverse transcriptase, 5 mM $MgCl_2$ and 2.5 μM oligo $d(T)_{16}$ primer. cDNA synthesis was carried out at 42 °C for 15 min. For amplification, 10 μl of cDNA was added with the PCR buffer, 2.5 U AmpliTaq DNA polymerase and amplified with a pair of primers from human CD4; β-actin cDNAs were used as the control for 30 cycles (Clontech Laboratories, Inc., Palo Alto, CA). Amplified DNA fragments were separated in a 2% agarose electrophoresis gel and transferred onto nitrocellulose membranes. The filter was hybridized with non-radio-labeled probe pT4B (ARRRP, Bethesda, MD). For the final detection and probe labeling, the Genius non-radioactive nucleic acid labeling and detection system (Boehringer Mannheim, Indianapolis, IN) was used according to the manufacturer's instruction.

Polymerase chain reaction and Southern blot detection of proviral sequences

Cells were trypsinized and washed with phosphate buffered saline (PBS). A total of 1×10^6 cells were lysed with 1% Triton X-100 in 10 mM Tris buffer (pH 8.0). The DNA was extracted using an Oncor non-organic DNA extraction kit (Oncor, Gaithesburg, MD). The primers for the amplification reaction, SK38/39 (Perkin Elmer Cetus, Norwalk, CT), were derived from a highly conserved region of the 136 base-pair fragment within the gag gene. One hundred μL of reaction mixture contained 10 μl chromosomal DNA (approximately 0.1 μg DNA), 1 μM each of primers, 200 μM each of dNTP, 10 mM Tris-HCl pH 8.0, 50 mM KCl, 2.5 mM $MgCl_2$, and 2.5 units of Taq polymerase. After incubation at 95 °C for 5 min, 40 cycles of denaturation, primer annealing, and chain elongation were performed. After amplification, sample DNA was electrophoresed in a 3% agarose gel and transferred onto nitrocellulose membranes (Schleicher & Schuell, NH). Filters were incubated for 1 h at 65 °C in a prehybri-

dization solution and hybridized to a ^{32}P-labeled, nick-translated HIV full-length probe (Oncor, Gaithesburg, MD) by incubation at 65 °C overnight. Filters were washed twice with 2 × SSC, 0.1% SDS solution for 15 min at 55 °C, then once with 0.5 × SSC, 0.1% SDS for 15 min at 65 °C and exposed overnight to Kodak X-Omat-AR film at −70 °C.

Electron microscopy

For electron microscopy ME180 cells were seeded in 24-well tissue culture plates (Becton Dickinson, Bedford, NJ) at a density of 4×10^5 cells/well and cultured for 24 h at 37 °C in a 5% CO_2 incubator. 10^5 HIV-1 infected mitomycin C treated monocytes, were co-incubated with the epithelial cells for 1 to 3 h. Cells were fixed and processed as previously described [23]. Thin sections were cut parallel to the monolayer.

Results

CD4 mRNA expression in ME180 cells

We have previously shown that ME180 cells are similar in appearance to cervical and vaginal epithelial cells in vivo [23]. Immunocytochemical and Western blot analysis were carried out to determine whether ME180 cells expressed CD4. Although these data suggested that ME180 cells do not express CD4, the sensitivity of both techniques is limited. Thus, low level expression could not be ruled out [23]. We present here RT-PCR and Southern blot analysis of mRNA from ME180 cells. MOLT-4 transformed T-cells and HT-6C cells (CD4 transfected HeLa cells) were used as positive controls. Hela cells, and I407 cells (CD4-negative epithelial cells) were used as negative controls. Our results indicated that the ME180 cell line does not express CD4 mRNA (Fig. 1a, b).

Immunocytochemistry

The purity of the HIV-infected monocyte preparation was determined by examination by phase microscopy and immunocytochemistry. Monocytes can be distinguished from the PBMCs because they are larger. The monocyte preparation consisted of more than 95% of the larger cells (Fig. 2). To confirm that these were monocytes we stained all cells (monocytes and PBMCs) with monoclonal antibodies to CD68 and CD14. Monocytes stained but the smaller cells in the PBMC fraction did not (Fig. 2).

Ultrastructure of monocyte-epithelial and interactions

We have previously shown that HIV-infected transformed T-cells and monocytes adhere to a variety of different types of epithelial cell lines. Adherence triggered a rapid shedding of virus onto the epithelium [20–23]. Primary activated and HIV-infected monocytes interact with ME180 cells in a different way. Only rarely were primary monocytes observed on the surface of the epithelium. Instead, an hour after they were added to confluent monolayers, HIV-infected monocytes were seen between epithelial cells (Fig. 3a–d). Primary HIV-infected monocytes extended a single pseudopod from which HIV virions

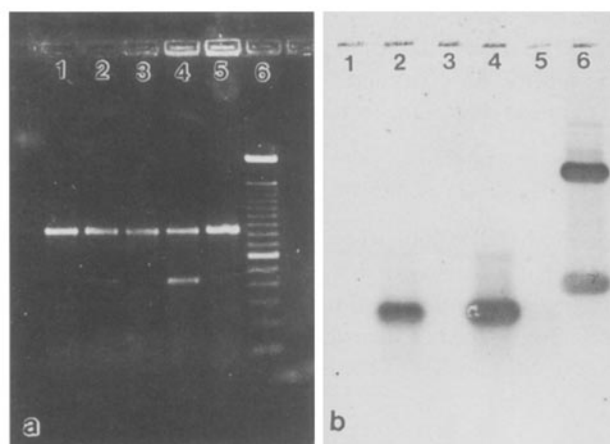

Fig. 1. **a** Agarose gel electrophoresis of PCR products from different cell lines. *1* HeLa, *2* HT-6C (CD4-transfected HeLa), *3* ME180, *4* MOLT4, *5* I407, *6* MW markers. **b** Blot of the gel in 1a shows the hybridization of probe pT4B with PCR amplification. HT-6C and MOLT4 cells, known to express CD4, are positive. The other cell lines do not express detectable levels of CD4

were seen to be budding (Figs. 3b, d). We have seen dozens of primary monocytes between epithelial cells shedding virus from what appeared to be a pseudopod.

Mitomycin C treatment

Mitomycin C had a dose dependent effect on killing activated HIV-infected monocytes. When a dose of 200 µg was employed, no viable cells could be detected with the MTT assay at 48 h (Fig. 4). This is the dose that we used in the studies described here. We examined cultures each day for 5 days. No viable cells were seen at this dose. Similar observations have been obtained by other laboratories [2, 12]. Since the assay was performed on the 5th day, we are confident that mononuclear cells were no longer present.

Polymerase chain reaction and Southern blot detection of proviral sequences

Polymerase chain reaction and Southern blot analysis was carried out to confirm that ME180 cells which had been co-cultured with HIV-1_{bal} infected monocytes were productively infected. We subcultured these cultures for three passages following the week after coculture. Among the controls we used were HeLa cells or CD4 transfected HeLa cells which had been co-incubated with HIV-infected monocytes. An HIV infected T-cell line (ACH2) was used as a positive control. The (131 MW) PCR products were observed only in the ME180 cells which had been co-cultured with the HIV-1_{bal} infected monocytes and the HIV-infected T-cell lines (Fig. 5).

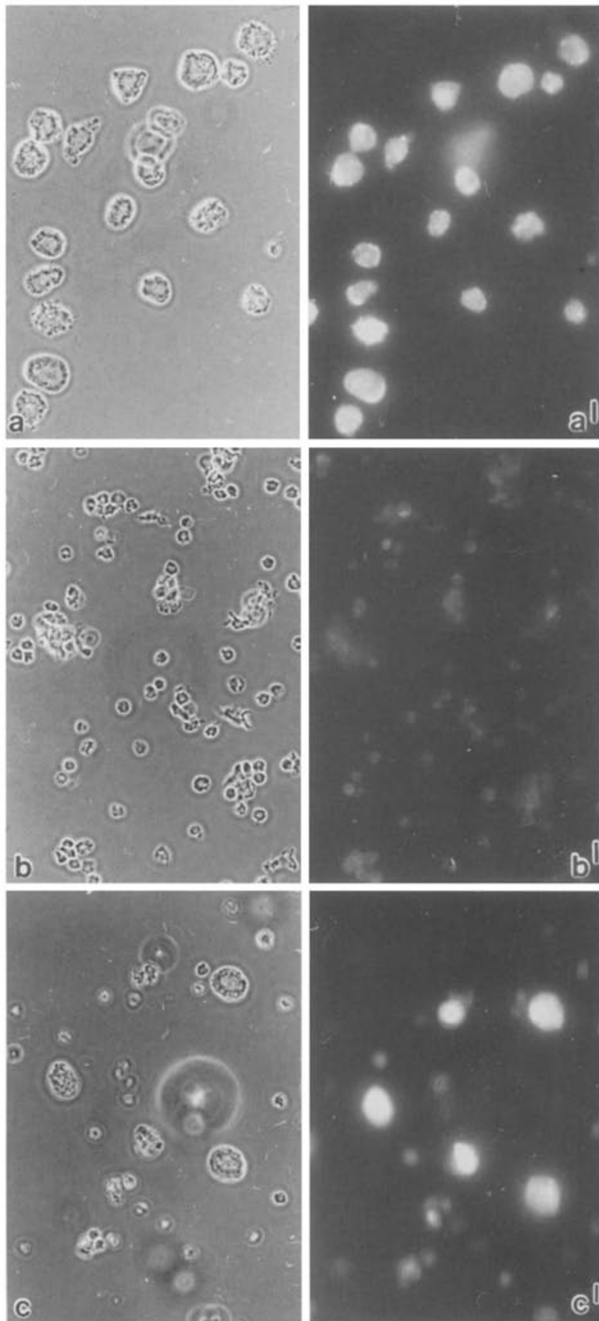

Fig. 2. Phase contrast and corresponding immunofluorescence photomicrographs of cells stained with CD68. **a**, **a'** Monocytes, **b**, **b'** PBMCs, **c**, **c'** mixture of monocytes and PBMCs. Monocytes are large and CD68 positive. Most PBMCs are smaller and CD68 negative

Infection assay

One week after initial co-culture, the level of HIV production by infected ME180 cells was assayed by a p24 ELISA of the medium in which the epithelial cells had been cultured for the previous 24 h. The level varied between 150 and 200 pg/ml

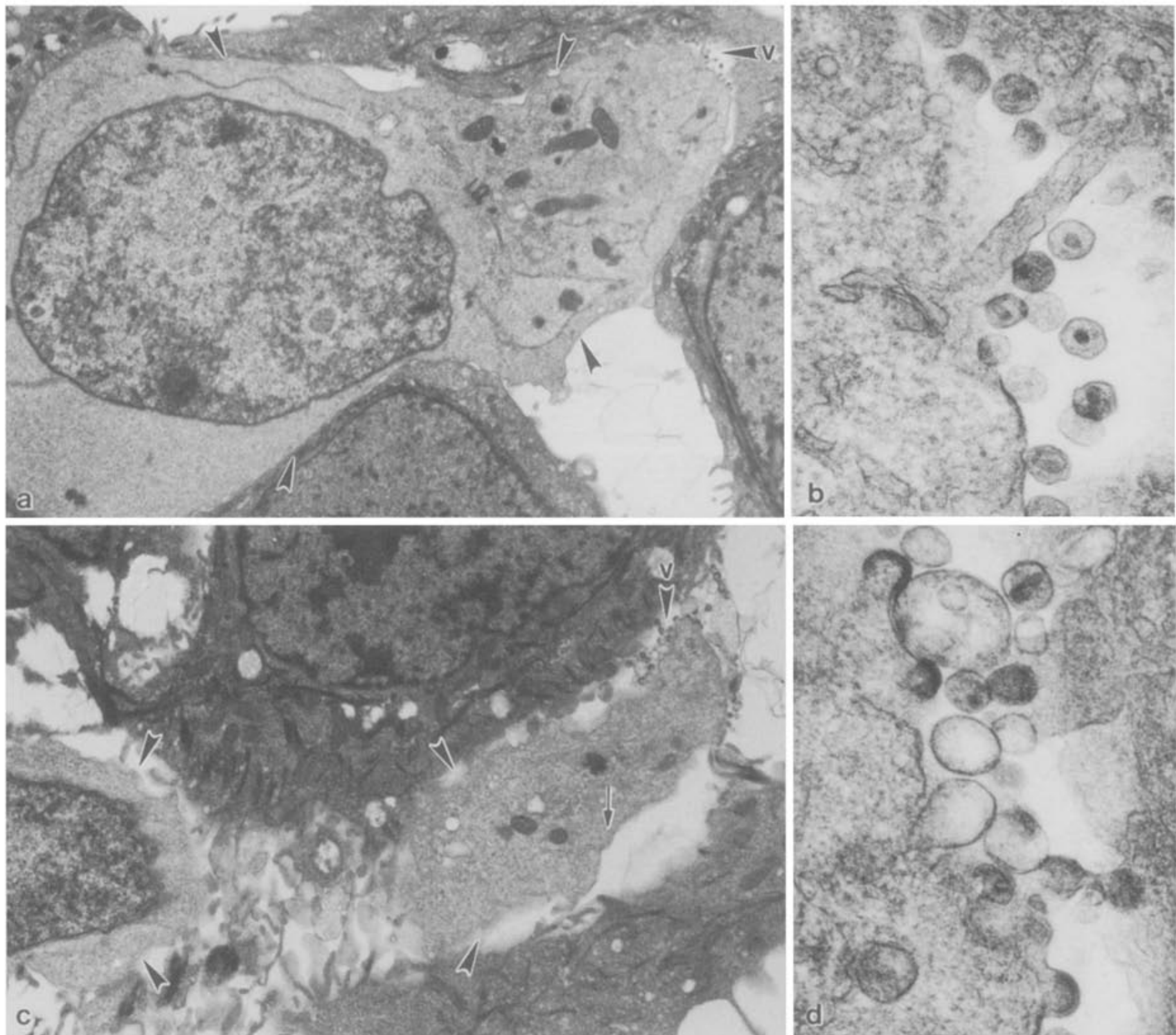

Fig. 3a–d. Transmission electron micrographs of sections cut parallel to the base of the ME180 epithelium (**b** and **d** are enlargements of a region of **a** and **c**, respectively). Cells were fixed 1h after the addition of HIV-infected monocytes strain P1–2. Monocytes (indicated by arrows) are observed between (darker staining) epithelial cells. Budding viruses and mature virions (*V*) are seen exclusively on a single pseudopod of the monocyte on the right side of Figs. 1a and 1c. **a**, **c** × 7000, **b**, **d** × 64000

of p24 per 2×10^6 cells. p24 production reached peak levels at 5 to 7 days after the addition of the virus.

Blocking studies

HIV-infected monocytes appeared to migrate between epithelial cells and secrete virus directly onto the surface of the epithelial cells. We reasoned that since virus

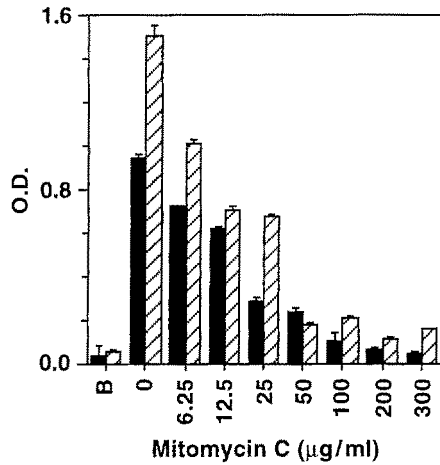

Fig. 4. Effect of various concentrations of mitomycin C on viability of HIV-infected activated monocytes as measured by the MTT assay. At 200 μg, the dose employed in this study, no viable cells were detected at 48 h; 24 h (solid bars), 48 h (hatched bars), Background fluorescence (*B*)

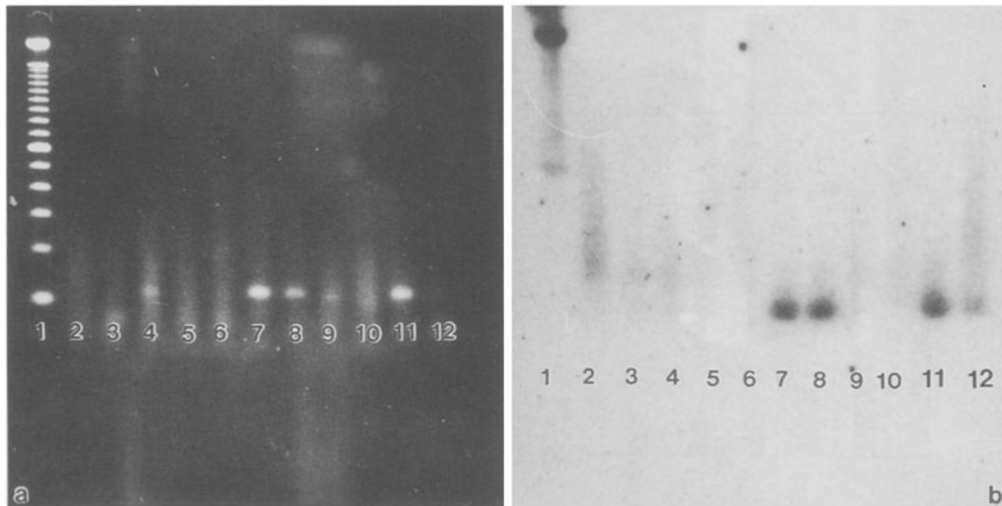

Fig. 5. **a** Agarose gel of PCR products, **b** corresponding Southern blot. *1* 100 bp molecular weight standards. *2* ME180 cells. *3* HeLa cells. *4* HeLa-CD4 cells. *5* HeLa cells that have been cocultured with HIV-1_{bal} infected monocytes. *6* HeLa cells that have been cocultured with supernate from HIV-1_{bal} infected monocytes. *7* ME180 cells that had been cocultured with HIV-1_{bal} infected monocytes and subcultured for 2 passages. *8* ME180 cells that had been cocultured with HIV-1_{bal} infected monocytes and subcultured for 3 passages. *9* HeLa-CD4 cells that had been cocultured with HIV_{bal} infected monocytes. *10* ME180 cells that had been cocultured with supernate from HIV-1_{MN} infected 89 cells. *11* 10^3 ACH2 cells. *12* 10^2 ACH2 cells

is secreted into the space between the target cell and the monocyte, there might not be sufficient time or access for antibody to block infection even if it were present. However, this is not the case. Patient serum was highly effective in blocking infection (Fig. 6). Rabbit polyclonal antibody to gp120 did not inhibit

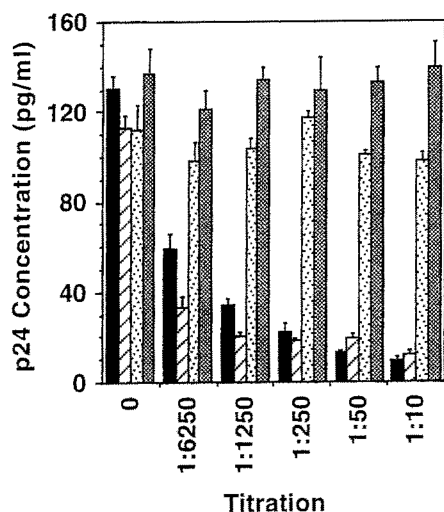

Fig. 6. Twenty-four hour accumulation of virus, strain P1–2, in medium, as measured by p24 ELISA. Solid and hatched bars represent two preparations of pooled patient antisera. These antisera are highly effective in blocking infection. Rabbit polyclonal antisera to HIV/IIIB (bars with parallel lines) does not block infection by these clinical isolates. Control human sera (bars with dots) did not block infection. Error bars represent standard deviation between three wells

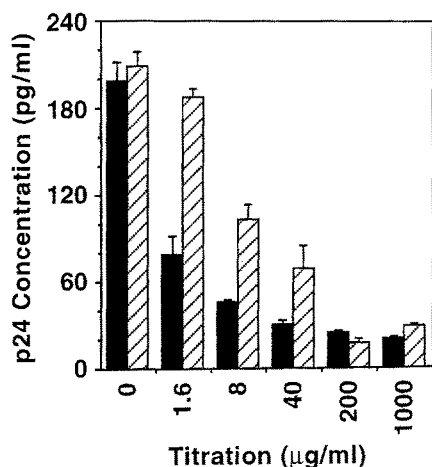

Fig. 7. Dextran sulfate (solid bar) and heparin (hatched bar) block transmission of HIV, strain P1–2, at low concentrations. Dextran sulfate is more effective that heparin

infection, but this is not surprising as the antibody is directed towards gp120 of the lab strain HIV-IIIB (Fig. 6).

A number of investigators have presented evidence that several sulfated polysaccharides inhibit infection of T-cells by laboratory strains of HIV at concentrations of less than one to a few μg/ml [3–6, 10, 13]. We have previously reported that some sulfated polysaccharides significantly inhibit T-cell mediated infection of BeWo (trophoblast derived) [20] and ME180 cell lines (21) in doses of less than 1 μg/ml. Dextran sulfate and heparin are also effective in μg/ml concentrations in the in vitro model described here (Fig. 7). This is clearly a specific effect on infection as the cytotoxic dose of dextran sulfate and heparin is 1000 times this dose [17].

Discussion

In this paper, we have presented evidence that epithelia derived from the human cervix can be productively infected by coculture with primary mononuclear cells that have been infected with primary isolates of HIV. We had previously presented evidence that the ME180 cell line can be infected by cell-cell contact [21, 23]. There can be little doubt that the virus that was measured by p24 ELISA a week after the donor HIV-infected monocytes were added was secreted by the ME180 cells, since no monocytes were viable two days after mitomycin C treatment. Blocking studies provided support for this as the blocking agent was added for a relatively brief period of time a week before virus was measured in the medium. We are confident that residual virus was not measured, as cultures were washed repeatedly after monocytes were removed and each day for 6 days before medium was collected for ELISA. To further confirm that ME180 cells were productively infected, we subcultured them for three passages and used PCR and Southern blot analysis to detect the proviral DNA in the cells.

ME180 cells appear very similar to epithelial cells of the ectocervix and vagina. As these epithelia are stratified, cells on the surface of the epithelium are not in contact with the basement membrane. Infection could theoretically occur either by emigration of mononuclear cells through the epithelium or by progressive infection of underlying epithelial cells by virions produced by the epithelial cell above it.

One of the isolates employed, P1–2 [9], may be particularly relevant to natural transmission, as it was isolated from a patient in the acute phase of infection, who was infected during sexual contact [8]. The observation that primary monocytes infected with primary isolates of HIV can infect ME180 cells supports the hypothesis that the CD4-negative epithelia at the portal of entry can serve as a primary target of HIV-infection. Until now this theory was based on studies employing transformed mononuclear cells and lab strains of HIV.

Co-culture with both HIV-infected transformed mononuclear cells [21, 23] and HIV-infected primary monocytes results in productive infection of the epithelium. However, the interaction between ME180 cells and HIV-infected primary monocytes is different from that previously described between these epithelial cells and HIV-infected transformed monocytes or lymphocytes. Rather than remaining adherent to the surface of the epithelium, HIV-infected primary monocytes progress between epithelial cells while shedding virus from their leading pseudopod. These results suggest the possibility that there might be conditions under which activated, HIV-infected monocyte/macrophages in semen or female genital tract secretions could emigrate between epithelial cells and perhaps even enter the lamina propria. Movement of mononuclear cells between epithelial cells occurs under certain conditions in vivo, for example, in the case of intraepithelial lymphocytes, white blood cell emigration from capillaries [16], and movement of leukocytes into the uterus or vagina [19]. The question of whether, under some conditions, HIV-infected monocytes are capable of moving out of the vaginal vault into the connective tissue merits further study.

The results of the antibody blocking experiments presented here are especially encouraging. If the model is an accurate representation of sexual transmission, then antibody present in genital tract secretions may be able to inhibit transmission of HIV. The observation that a few micrograms of dextran sulfate blocks infection is also encouraging as it suggests that a topical formulation of a sulfated polysaccharide may be effective in inhibiting sexual transmission of HIV as we had previously proposed, based on our studies of transformed T-cells [17, 23].

Acknowledgements

We are grateful to Drs. Ruth Connor and David Ho for providing patient isolates of HIV. We would like to thank Vanaja Zacharopoulos and Rachael Pearce-Pratt for their help and suggestions. We are also grateful to Christine Burillo for technical assistance. This research was supported by Grant RO1 AI37793 from The National Institute of Allergy and Infectious Diseases, and grant U54HD29990 from the National Institutes of Child Health and Human Development.

References

1. Current and future dimensions of the HIV/AIDS pandemic. In: Global Program on AIDS, Document WHO/GPA/RES/SF1/92.1., 1992, p. 8
2. Current Protocols in Immunology (1994) J. Wiley, New York, pp. 3.12.9
3. Aoki T, Kaneko Y, Stefanski MS, Nguyen T, Ting RCY (1991) Curdlan sulfate and HIV-1 I. In vitro inhibitory effects of curdlan sulfate on HIV-1 infection. AIDS Res Hum Retroviruses 7: 409–415
4. Baba M, Nakajima M, Schols D, Pauwels R, Balzarini J, De Clercq E (1988) Pentosan polysulfate, a sulfated oligosaccharide, is a potent and selective anti-HIV agent in vitro. Antiviral Res 9: 335–343
5. Baba M, Schols D, Pauwels R, Balzarini J, Nakajima M, De Clercq E (1988) Sulfated polysaccharides as potent and selective anti-HIV agents in vitro. Proceedings of the Fourth International Conference on AIDS, Stockholm, 3542 (Abstract)
6. Baba M, Snoeck R, Pauwels R, De Clercq E (1988) Sulfated polysaccharides are potent and selective inhibitors of various enveloped viruses, including herpes simplex virus, cytomegalovirus, vesicular tomatitis virus, and human immunodeficiency virus. Antimicrob Agents Chemother 32: 1742–1745
7. Chomcznski P, Sacchi N (1987) Single-step isolation of RNA isolation by acid guanidinium thiocyanate-phenol-chloroform extraction. Anal Biochem 162: 156–159
8. Connor RI, Mohri H, Cao Y, Ho DD (1993) Increased viral burden and cytopathicity correlate temporally with CD4+ T-lymphocyte decline and clinical progression in human immunodeficiency virus type 1 infected individuals. J Virol 67: 1772–1777
9. Connor RI, Notermans DW, Mohri H, Cao Y, Ho DD (1993) Biological cloning of functionally diverse quasispecies of HIV-1. AIDS Res Hum Retroviruses 9: 541–546
10. De Clercq E (1986) Chemotherapeutic approaches to treatment of the acquired immune deficiency syndrome (AIDS). J Med Chem 29: 1561–1569
11. Gendelman HE, Orenstein JM, Martin MA, Ferrua C, Mitra R, Phipps T, Wahl LA, Lane HC, Fauci AS, Burke DS, Skillman D, Meltzer MS (1988) Efficient isolation and propagation of human immunodeficiency virus on recombinant colony-stimulating factor 1 treated monocytes. J Exp Med 167: 1428
12. Ho DD, Rota TR, Hirsch MS (1984) Infection of human endothelial cells by human T-lymphotropic virus type I. Proc Natl Acad Sci USA 81: 7588–7590

13. Ito M, Baba M, Hirabayashi K (1989) In vitro activity of mannan sulfate, a novel sulfated polysaccharide, against human immunodeficiency virus type 1 and other enveloped viruses. Eur J Clin Microbiol Infect Dis 8: 171–173
14. Moore JP, Cao Y, Qing L, Sattenau QJ, Pyati J, Koduri R, Robinson J, Barbas CF 3rd, Burton DR, Ho DD (1995) Primary isolates of human immunodeficiency virus type 1 are relatively resistant to neutralization by monoclonal antibodies to gp120, and their neutralization is not predicted by studies with monomeric gp120. J Virol 69: 101–109
15. Mosmann T (1983) Rapid colorimetric assay for cellular growth and survival: application to proliferation and cytotoxicity assays. J Immunol Methods 65: 55–63
16. Muller WA, Weigl SA, Deng X, Phillips DM (1993) PECAM-1 is required for transendothelial migration of leukocytes. J Exp Med 178: 449–460
17. Pearce-Pratt R, Phillips DM (1993) Studies of adhesion of lymphocytic cells: implications for sexual transmission of human immunodeficiency virus. Biol Reprod 48: 431–445
18. Phillips DM (1994) The role of cell-to-cell transmission in HIV infection. AIDS 8: 1–13
19. Phillips DM, Mahler S (1977) Leukocyte emigration and migration following mating in rabbit. Anat Res 189: 45–59
20. Phillips DM, Tan X (1992) Mechanism of trophoblast infection by HIV. AIDS Res Hum Retroviruses 9: 1697–1705
21. Phillips DM, Tan X, Pearce-Pratt R, Zacharopoulos VR (1995) An assay for HIV infection of cultured human cervix-derived cells. J Virol Methods 52: 1–13
22. Phillips DM, Zacharopoulos VR, Tan X, Pearce-Pratt R (1994) Mechanisms of sexual transmission of HIV: does HIV infect intact epithelia? Trends Microbiol 2: 454–458
23. Tan X, Pearce-Pratt R, Phillips DM (1993) Productive infection of a cervical epithelial cell line with human immunodeficiency virus: Implications for sexual transmission. J Virol 67: 6447–6452
24. Wolff H, Anderson DJ (1988) Immunohistologic characterization and quantitation of leukocyte subpopulation in human semen. Fertil Steril 49: 497–504
25. Zhu T, Mo H, Wang N, Nam DS, Cao Y, Koup RA, Ho DD (1993) Genotypic and phenotypic characterization of HIV-1 in patients with primary infection. Science 261: 1179–1181

Authors' address: Dr. D. M. Phillips, The Population Council, 1230 York Avenue, New York, NY 10021, U.S.A.

Received November 6, 1995