Immunology 1995 **85** 475–484

Comparative investigation of Langerhans' cells and potential receptors for HIV in oral, genitourinary and rectal epithelia

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SUMMARY

Human immunodeficiency virus (HIV) is commonly transmitted, during homosexual and heterosexual intercourse, through the rectal and cervicovaginal mucosa, foreskin and urethral epithelia. However, there is uncertainty about HIV transmission through the oral mucosa by oral sex. We have carried out a comparative immunohistological investigation of primate oral, cervicovaginal, foreskin, urethral and rectal epithelia for potential HIV receptors. We investigated epithelial tissues for CD4 glycoprotein, which is the principal receptor for HIV, Fc receptors of IgG for binding HIV–IgG antibody complexes, and HLA class II, which might enable HIV-bound $CD4^+$ cells to gain access to the epithelial cells. CD4 glycoprotein was not found in oral, foreskin, urethral, vaginal or rectal epithelial cells, although $CD4^+$ mononuclear cells were present in the lamina propria of each epithelium. FcγII and FcγIII receptors were found in urethral, endocervical and rectal epithelia, and FcγIII and FcγI receptors in the foreskin. However, Fcγ receptors were not found in oral epithelium (buccal, labial, lingual or palatal) and only FcγIII receptors were detected in the gingival epithelial cells. HLA class II antigen was also not detected in foreskin, oral or rectal epithelium, but it was expressed by endocervical cells from most human specimens and in male urethral epithelia of non-human male primates. Langerhans' cells were found in all epithelia except those of the urethra and rectum, and they can express CD4 glycoprotein, Fcγ receptors and HLA class II antigen. The mean number of Langerhans' cells expressing CD4 in the upper third of oral epithelium was significantly lower compared with vaginal epithelium or foreskin. The HIV-binding V1 domain of CD4 was significantly decreased in Langerhans' cells present in oral compared with vaginal epithelium. The results suggest that the foreskin in uncircumcised men and the cervicovaginal epithelium in females might become infected via the $CD4^+$ Langerhans' cells. However, urethral infection might be mediated by HIV–antibody complexes binding to urethral epithelial Fcγ receptors. The paucity of Langerhans' cells expressing the V1 domain of CD4, the absence of Fcγ receptors, and a lack of expression of HLA class II antigens in most oral epithelial cells, argue against transmission of HIV through the normal intact oral mucosa.

INTRODUCTION

Mucosal transmission of human immunodeficiency virus (HIV) by the vaginal and rectal mucosa in heterosexual and homosexual subjects may be responsible for 70–80% of acquired immune deficiency syndrome (AIDS).[1–4] The foreskin and/or urethra might also become infected if exposed to HIV-infected cervical secretions during heterosexual intercourse or by direct contact with infected rectal tissues during homosexual intercourse.[2,5–9] However, transmission of HIV via the oral epithelium during oral sex has not been established.[10–12] There is no convincing evidence that HIV in infected seminal fluid[13] ejaculated during oral sex gains entry through the oral mucosa.[14] Furthermore, there is considerable doubt whether intimate kissing between seropositive and seronegative subjects may lead to oral transmission of HIV, although this might be accounted for by the low frequency of isolation and low concentration of HIV in saliva.[15]

Epidemiological studies conducted in Africa have shown that lesions that disrupt the integrity of the genital epithelium, such as those that result from sexually transmitted disease, may facilitate transmission of HIV.[7] The most accessible part of the male genitourinary tract in sexually transmitted diseases is the penile urethra, which is lined by pseudostratified columnar epithelium, and the foreskin and the head of the penis, lined by stratified squamous epithelium. The efficiency of female to male heterosexual transmission of HIV is significantly increased by the presence of foreskin[7,8] and uncircumcised homosexual men have a twofold increased risk of HIV infection.[9] In addition,

Received 22 December 1994; revised 2 March 1995; accepted 7 March 1995.

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uncircumcised men have an increased risk of genital ulceration, which facilitates HIV transmission.[8,16,17] Indeed, simian immunodeficiency virus (SIV) has been transmitted to male macaques by placing cell-free virus onto the foreskin and urethra.[18,19] Furthermore, male genital immunization in rhesus macaques has been achieved by topical urethral immunization with a recombinant SIV gag p27.[20]

CD4 glycoprotein is the main receptor for HIV, and targets for HIV appear to be cells expressing the 55 000 MW CD4 glycoprotein, such as T cells, macrophages, dendritic and Langerhans' cells.[21,22] The V1 domain of the CD4 glycoprotein binds the HIV gp120 envelope glycoprotein.[23–25] The other three domains of CD4 may be involved in cell to cell fusion (syncytium formation).[26,27] HIV has also been detected in Langerhans' and dendritic cells of patients with AIDS,[28,29] although others have been unable to confirm this.[30,31] SIV infects Langerhans' cells in the female genital tract epithelium of rhesus monkeys.[32] Recently, SIV-infected Langerhans' cells were found in the lamina propria and stratified squamous epithelium of the foreskin.[33] Transmission of HIV through rectal or cervicovaginal epithelium may occur by either cell-free or cell-associated virus, as both have been demonstrated in seminal fluid.[13,34] Cell-free HIV-1 may directly infect $CD4^+$ Langerhans' cells, as their processes can reach the mucosal surface.[35] An alternative pathway for HIV transmission might be HIV–IgG antibody complexes in seminal fluid, which bind to Fc receptors of IgG in rectal or endocervical epithelial tissues.[35,36]

There is a paucity of information about the immune system of the genitourinary tract, and the target cells of HIV infection of the male genitourinary tract are unclear. There is also considerable doubt about HIV transmission through oral epithelium. We have therefore investigated the foreskin, male urethral epithelium and oral epithelium for the expression of potential receptors for HIV. CD4 glycoprotein, Fc receptors for IgG, HLA class II and Langerhans' cells were compared between foreskin, male urethral, oral, cervicovaginal and rectal epithelia. In addition, we studied the distribution of immunocompetent cells in the foreskin and male urethra. Indeed, $CD4^+$ Langerhans' cells were increased in the foreskin, which also showed FcγI and FcγIII receptors. The virtual absence of Fcγ receptors, the lack of expression of HLA class II antigens in oral epithelial cells, and the paucity of Langerhans' cells near the mucosal surface, argue against transmission of HIV through the intact oral epithelium.

MATERIALS AND METHODS

Collection and preparation of tissue sections

Oral mucosal biopsies were obtained from 16 healthy subjects (nine male and seven female; age range 15–50 years; by courtesy of Mr P. Robinson, Department of Oral Surgery, Guy's Hospital, London, UK). These included four buccal, four vestibular, five gingival, two hard palate and one lingual specimens. Foreskin was obtained during circumcision from seven infants (age range 3 weeks to 7 months), and three older specimens were from 7-, 8- and 36-year-old subjects. Urethral tissue was obtained from seven human male fetuses (16–20 weeks). In addition, biopsies of oral mucosa were taken from six rhesus monkeys, from the tongue, gingiva and soft palate, and urethral tissue was taken from eight adult rhesus monkeys at autopsy. Biopsies of rectal mucosa were obtained from Mr M. Jourdan (Department of Surgery, Guy's Hospital, London, UK) for diagnostic purposes, and 18 specimens that appeared clinically and histologically normal were selected. In addition, normal cervicovaginal tissues showing no macroscopic cervical abnormalities were obtained from Professor M. Chapman (Department of Obstetrics and Gynaecology, Guy's Hospital, London, UK) from eight patients undergoing hysterectomies, six of whom were premenopausal and two were postmenopausal. The rectal and cervicovaginal tissues were obtained for earlier studies, and have been reported previously.[35,36] Blocks of tissues were embedded in OCT compound (Miles, Elkhart, IN), frozen in liquid nitrogen and then stored at −70°. Serial cryostat sections, 6 μm thick were cut, air dried for 1 hr, and sections of all samples were stained with haematoxylin and eosin, and with a series of monoclonal antibodies (mAb).

Monoclonal antibodies

A series of mAb were used in this study against CD4 glycoprotein and its different domains. ADP318 (domain 1-NH_2 terminal), ADP302 (domain 1-COOH terminal), ADP3015 (domain 3) and ADP359 (domain 4) were provided by the Medical Research Council (AIDS Directed Programme, London, UK) and mAb 5A8 (domain 2) was kindly provided by Dr Q. Sattentau (Centre d'immunologie de Marseille, Luming, France). The antibody class, molecular weight, source and tissue specifications of the above mAb and those against Fc receptors for IgG, Langerhans' cells, dendritic cells, HLA-DR, HLA-DP, HLA-DQ, CD8 T cells, macrophages and B cells are given in Table 1.

Indirect immunoperoxidase technique

The indirect immunoperoxidase technique was applied, using the avidin–biotin–peroxidase complex method.[37] The sections were first incubated with the mAb for 75 min after fixation with acetone for 10 min, and washed twice with Tris-buffered saline. Rabbit anti-mouse IgG or goat anti-mouse IgM biotin conjugate was used as a second layer for 1 hr, followed by incubation with the streptavidin–horseradish–peroxidase complex (Dakopatts, Glostrup, Denmark) for 30 min, at room temperature. After further washing, the sections were developed in a solution containing hydrogen peroxide and 3-3 diaminobenzidine (DAB) tetrahydrochloride (Sigma, Poole, UK). Sections were counterstained with haematoxylin, dehydrated and mounted in a synthetic mountant. Negative controls were performed by omitting the primary antibody with all mAb and with all sections. The optimal dilution for each mAb was predetermined on sections of tonsil, which were also used as positive controls.

Morphometric analysis of epithelial thickness and of Langerhans' cells

A Zeiss MOP-Videoplan image analysis system (64K Z80 microprocessor), attached to a Zeiss microscope with a digitizing tablet, was used for these measurements.

Epithelial thickness. The distance between the basement lamina and the free surface of the epithelium was measured for oral, rectal and vaginal epithelia. Heamatoxylin and eosin-stained sections were first scanned using a Zeiss Microscope with a ×20 objective. The thickness of rectal epithelium was determined by 8-11 measurements of the surface and crypt epithelium from each biopsy. The measurement of vaginal or

Table 1. Monoclonal antibodies used in this study

Name	Cluster designation	Clone no.	Class	Molecular weight of antigen (MW) $\times 10^{-3}$	Optimal dilution	Source	Specificity in normal tissues
OKT4a	CD4		IgG2	60	1:10	Ortho-mune	Helper/inducer T cell
Leu-3a	CD4	SK3	IgG1	55	1:10	Becton Dickinson	Helper/inducer T cell
L200	CD4		IgG1	55	1:10	Dr C. Miller	Helper/inducer T cell
ADP302	CD4 (D1-COOH terminal)		IgG1	55	1:50	ADP (MRC)	Helper/inducer T cell
ADP318	CD4 (D1-NH_2 terminal)		IgG1	55	1:50	ADP (MRC)	Helper/inducer T cell
5A8	CD4 (D2)		IgG	55	1:50	Dr Q. Sattentau	Helper/inducer T cell
ADP3015	CD4 (D3)		IgG	55	1:50	ADP (MRC)	Helper/inducer T cell
ADP359	CD4 (D4)		IgG	55	1:50	ADP (MRC)	Helper/inducer T cell
HTA1-C1	CD1	NA1/34 HLK	IgG2	49	1:20	Sero-Lab	Langerhans' cells in the skin and rare dendritic cells in the lymph nodes and thymus
Leu-6	CD1	SK9	IgG2b	49	1:10	Becton Dickinson	Langerhans' cells in skin
RFDR1			IgM	28/33	1:5	Royal Free Hospital	HLA-DR
HLA-DR		L243	IgG2a	27–36	1:30	Becton Dickinson	HLA-DR
HLA-DP		B7/21	IgG1	28–33	1:30	Becton Dickinson	HLA-DP
HLA-DQ			IgG	28–33	Neat	Dr J. Bodmer	HLA-DQ
FcγRI 22	CD64		IgG1	70	1:30	Medarex	Monocytes, macrophages
32.2					1:30	Medarex	Monocytes, macrophages
FcγRII	CD32	2E1	IgG2a	48	1:10	Immunotech	Granylocytes, monocytes
FcγRIII (Leu-11b)	CD16	GO22	IgM	50–70	Neat	Becton Dickinson	Neutrophil—natural killer cells—epithelial cells, peripheral blood, lymphocytes
3G8	CD16		IgG1	50–70	1:30	Medarex	Granulocytes
Macrophages	CD68	EBM1	IgG1	110	1:30	Dako	Human macrophages and react with macrophages and dendritic cells from monkey
Leu-2a	CD8	SK1	IgG1	32–43	1:30	Becton Dickinson	Cytotoxic/suppressor T cells
RFD1			IgM		1:5	Royal Free Hospital	Interdigitating (dendritic) cells and subpopulation of B cells
IF-5	CD20		IgG		1:5	Professor E. Clark (University of Washington)	B cells
CR2	CD21	HB-5	IgG2a	145	1:30	Becton Dickinson	B cells

Source locations: Ortho-mune (High Wycombe, UK); Becton Dickinson (Oxford, UK); Dr C. Miller (University of California, CA); Dr Q. Sattentau (Centre d'Immunologie de Marseilla, France); Sero-Lab (Sussex, UK); Royal Free Hospital (London, UK); Medarex (New Jersey, NJ); Immunotech (Marseille, France); Dako (High Wycombe, UK).

oral epithelium consisted of 15–20 readings at about equal intervals along the entire length of epithelium for (1) minimum epithelial thickness (shortest distance between the basement lamina at the tip of connective tissue papilla and the free epithelial surface), and (2) for maximum epithelial thickness. The results are expressed as the mean thickness ± SD.

Morphometric analysis of Langerhans' cells. CD1-stained sections were first scanned using a ×40 objective, and four to five areas of the epithelium were selected at about equal intervals along the entire length of the specimen, to count the $CD1^+$ Langerhans' cells, identified by a definable cell body. The area of epithelium was then measured by using a light cursor, to trace the outline of the area of the epithelium, within which the number of $CD1^+$ and serial sections of $CD4^+$-labelled cells were assessed. The results are expressed as the number of Langerhans' cells/mm^2 of the epithelium. Langerhans' cells were also counted in the upper third of the epithelium, which was traced with a light cursor, by using an eye piece graticule dividing the epithelium into three parts: upper, intermediate and basal.

RESULTS

Epithelial thickness

The mean thickness (± SD) of the oral epithelium was 263 ± 106 μm, and vaginal epithelium was of a similar

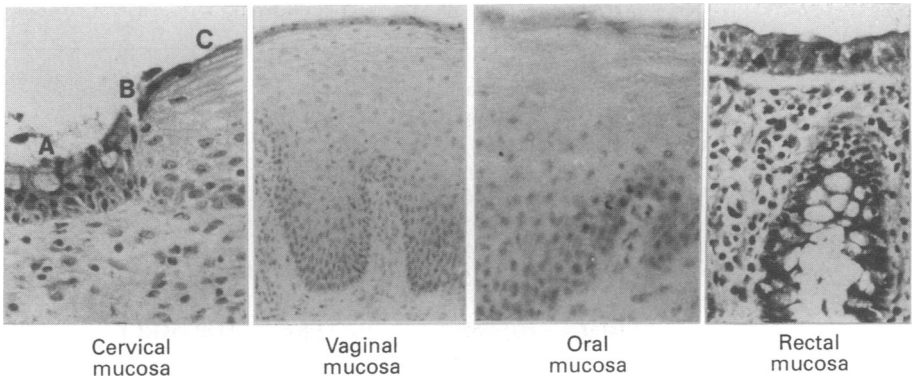

Figure 1. Comparative assessment of the thickness of vaginal, oral and rectal epithelial tissues (× 165); the measurements are given in the text. A, endocervix ; B, transformation zone; C, ectocervix.

thickness (215·5 ± 89·2 μm). In contrast, rectal epithelium was about one-tenth of the thickness of oral or vaginal epithelium, measuring 24·6 ± 9·7 μm (Fig. 1).

$CD4^+$ cells and Langerhans' cells in oral mucosa

CD4 glycoprotein was not detected in oral epithelial cells, but a number of $CD4^+$ mononuclear cells infiltrated the epithelium. $CD4^+$ mononuclear cells were detected in the lamina propria in all specimens examined, using Leu-3a and OKT4a mAb, and most of the cells were adjacent to the epithelium. In clinically normal human and macaque oral epithelium, Langerhans' cells were detected with mAb CD1 (Leu-6 and NA1/34) in all specimens examined (Fig. 2e). The mean number of Langerhans' cells was 13·9 ± 2·3 and a smaller proportion of CD1 cells was detected in seven out of eight specimens in the upper third of the epithelium (6·5 ± 0·7) (Table 2). There were regional variations in the distribution and density of Langerhans' cells in the oral mucosa; the dorsum of tongue showed a much higher number than found in the lingual surface or in the soft palate. In the gingival specimens, more Langerhans' cells were found in the oral than crevicular epithelium. These findings in human oral epithelium were confirmed in the corresponding macaque epithelia.

Some of the Langerhans' cells expressed CD4, which was detected by means of Leu-3a mAb (Fig. 2f), and the mean number was 5·9 ± 1·4/mm^2 (Table 2). Only a few $CD4^+$ Langerhans' cells were detected in the upper third of the epithelium in three out of eight specimens, with a mean number of 1·4 ± 0·7 (Fig. 4). Monoclonal antibodies recognizing different domains of CD4 were then used to find out if the HIV gp120 binding domain was found near or at the epithelial surface (Table 3). Langerhans' cells expressing the CD4 domain 1 were detected in the upper third of the epithelium in only one out of seven specimens of oral alveolar mucosa using mAb ADP318, and in one specimen of oral vestibular mucosa using mAb ADP302 (Table 3).

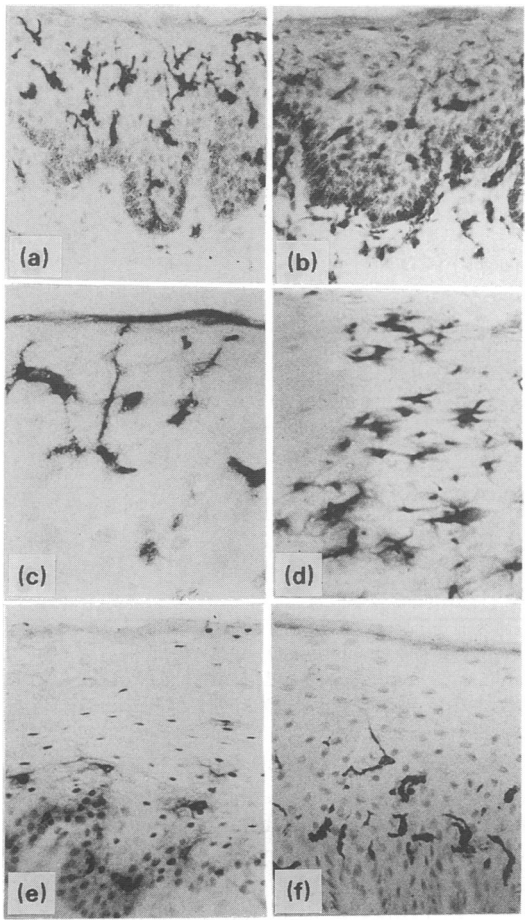

Figure 2. Comparative study of Langerhans' cells (LC) in foreskin, vaginal and oral epithelia expressing CD1 or CD4 glyocprotein. (a) Foreskin epithelium, $CD1^+$ cells (× 115); (b) foreskin epithelium, $CD4^+$ cells (× 115); (c) vaginal epithelium, $CD1^+$ cells (× 460); (d) vaginal epithelium, $CD4^+$ cells (× 460); (e) oral epithelium, $CD1^+$ (× 230); (f) oral epithelium, $CD4^+$ cells (× 230).

T cells, macrophages, B cells and Langerhans' cells in the foreskin

CD4 glycoprotein was not detected in the human foreskin epithelium using Leu-3a and OKT4 mAb. However, a large number of $CD4^+$ mononuclear cells was present in the lamina

Table 2. Comparison between adult oral, vaginal, ectocervical and foreskin (adult and infant) Langerhans' cells, expressing CD1 or CD4 (Leu-3a)

Location of Langerhans' cells	Oral (n = 8)		Vaginal (n = 8)		Ectocervical (n = 8)		Foreskin* (n = 7) 1–7 months		Foreskin (n = 3) 7–36 years	
	CD1	CD4	CD1	CD4	CD1	CD4	CD1	CD4	CD1	CD4
Whole epithelium										
No. of cells/mm^2										
Mean ± SEM	13·9 ± 2·3	5·9 ± 1·4	27·0 ± 3·6	12·9 ± 1·8	24·9 ± 3·0	13·4 ± 2·3	41·9 ± 4·6	25·2 ± 9·5	26·0 ± 5·7	12·0 ± 1·2
Range	5–26	1–11	9–40	7–20	19–41	7–22	22–56	8–36	16–35	9–13
Upper third of epithelium										
No. of cells/mm^2										
Mean ± SEM	6·5 ± 0·7	1·4 ± 0·7	17·4 ± 5·2	7·4 ± 2·2	13·2 ± 2·3	7·8 ± 2·1	44·9 ± 12·3	23·2 ± 4·7	38·7 ± 9·2	10·6 ± 0·8
Range	0–10	0–4	0–41	0–18	6–22	0–19	7–97	9–42	20–51	9–12

* Mucosal surface.

propria of all specimens examined, and a small number of $CD4^+$ cells was found to infiltrate the epithelium. Langerhans' cells expressing CD4 glycoprotein were found in basal, suprabasal and in the upper third of the epithelium (Fig. 2b). A small number $CD8^+$ cells were also found in the foreskin lamina propria in all specimens examined using Leu-2a mAb. In addition, small lymphoid follicles were found in the lamina propria adjacent to the epithelium, and most of the cells were $CD4^+$ cells, with a small number of $CD8^+$ cells. Macrophages were also detected in the foreskin lamina propria using mAb CD68, and so were B cells in all specimens using CD20 (IF-5) mAb, but not with CD21 mAb.

Langerhans' cells were detected in human foreskin epithelium using CD1-specific mAb NA1/34 and Leu-6 mAb in all 10 specimens examined (Fig. 2a). The foreskin could be differentiated morphologically into an outer skin surface and inner mucosal surface, by taking into account the absence of sebaceous gland, and the relative decrease in melanocytes and thickness of the epithelium of the inner mucosal surface. The mean number of $CD1^+$ Langerhans' cells of the inner mucosal surface of infants was 41·9 ± 4·6 cells/mm^2, and CD4 was expressed in a mean of 25·2 ± 9·5 cells/mm^2 (Table 2). However, foreskin from the 7–36-year subjects showed a lower number of Langerhans' cells, with a mean of $CD1^+$ cells of 26·0 ± 5·7, and of $CD4^+$ cells of 12·0 ± 1·2. Indeed, most of the Langerhans' cells were located in the upper third of the foreskin epithelium, with a mean number of $CD1^+$ Langerhans' cells in infants of 44·9 ± 12·3, and in the older group of 38·7 ± 9·2 cells/mm^2 (Table 2, Fig. 4). The corresponding number of Langerhans' cells expressing CD4 in infant foreskin was 23·2 ± 4·7, and in the older group 10·6 ± 0·8 cells/mm^2 (Table 2). Although we had difficulties with some specimens in differentiating the inner mucosal from the outer skin surface, this may not have affected the results as there was no significant difference in the number of $CD1^+$ or $CD4^+$ Langerhans' cells between the two surfaces. Monoclonal antibodies recognizing different domains of CD4 were then used and Langerhans' cells expressing domain 1 of CD4 were detected in seven or eight out of 10 specimens in the upper third of the epithelium, using mAb ADP318 or ADP302 (Table 3). Domain 3 expression in Langerhans' cells was not detected and the reason for this is not known, but it is unlikely to be due to denaturation of CD4 as $CD4^+$ mononuclear cells expressing domain 3 (ADP3015) were found in the lamina propria of the foreskin.

T cells, macrophages, B cells and Langerhans' cells in urethral epithelium

CD4 glycoprotein was not detected in urethral epithelium of human fetuses and adult macaques using L200 and OKT4 mAb. However, a few $CD4^+$ mononuclear cells were found infiltrating the epithelium in six out of eight adult macaques

Table 3. Immunohistological findings of Langerhans' cells expressing different domains of CD4 in the upper third of epithelium in human oral, foreskin vaginal and cervical specimens. The results are presented as the number of specimens reacting with the mAb tested

mAb	CD4 domain	Oral epithelium (n = 7) upper third of epithelium	Vaginal epithelium (n = 6) upper third of epithelium	Cervical epithelium (n = 6) upper third of ectocervical epithelium	Foreskin epithelium (n = 10) upper third of epithelium
ADP318	D1 (face NH_2 terminal)	1	5	4	8*
ADP302	D1 (face COOH terminal)	1	4	6	7
5A8	D2	1	5	4	6
ADP359	D4	1	4	5	4

Langerhans' cells did not react with the CD4 domain D_3 mAb ADP3015.

* Six specimens 1–7 months old and two specimens 7 and 36 years old, respectively.

Table 4. Comparison between CD1$^+$ Langerhans' cells and RFD1$^+$ dendritic-like cells in mucosal tissues

Mucosal tissue	Epithelium Langerhans' cells	Lamina propria dendritic cells*
Vaginal	10/10	1/7†
Ectocervical	14/14	2/7
Endocervical	9/14	2/7
Foreskin	10/10	2/10
Rectal	0/18	5/6
Urethral	0/8	6/8

* Only 6 to 10 specimens were examined for dendritic cells.
† Only two to four cells.

and in one out of seven fetal human urethra. A small number of CD4$^+$ mononuclear cells was found in the lamina propria of all macaque specimens examined, and three of them had lymphoid follicles in the submucosa of the urethra. These follicles were composed of CD4$^+$ and CD8$^+$ cells, and a few CD21$^+$ B cells. In addition, CD8$^+$ mononuclear cells were found in the lamina propria in all eight macaque specimens using Leu-2a mAb, and the number of CD8$^+$ T cells infiltrating the urethral epithelium was greater than the number of CD4$^+$ cells. However, CD8$^+$ cells were not detected in the urethral specimens of human fetuses. There was a moderate number of CD68$^+$ macrophages in the lamina propria in all macaque specimens and in some sections a few macrophages infiltrated the epithelium. B cells were not detected in urethral epithelium of human fetuses or adult macaques, using CD20 (IF-5 mAb), but a few B cells were detected in the lymphoid follicles using CD21 mAb in two out of eight macaque specimens. CD1$^+$ Langerhans' cells were not found in urethral epithelium of human fetuses and adult macaques.

Langerhans' cells in vaginal and cervical mucosa

The mean number of Langerhans' cells detected by CD1 mAb (Leu-6 and NA1/34) was significantly greater in vaginal ($P < 0{\cdot}02$) and ectocervical ($P < 0{\cdot}02$) epithelium than in oral epithelium (Fig. 2c). Langerhans' cells expressing CD4 were detected with mAb Leu-3a and OKT4a in all specimens of ectocervical and vaginal epithelia. The mean number of CD4$^+$ Langerhans' cells in the vagina was $12{\cdot}9 \pm 1{\cdot}8\,mm^2$, and in the ectocervix $13{\cdot}4 \pm 2{\cdot}3$ cells/mm^2 (Table 2). CD4$^+$ Langerhans' cells were also detected in seven out of eight specimens in the upper third of the epithelium (Fig. 2d), with $7{\cdot}4 \pm 2{\cdot}2$ cells/mm^2 in the vagina and $7{\cdot}8 \pm 2{\cdot}1$ cells/mm^2 in the ectocervix (Fig. 4). Furthermore, Langerhans' cells expressing domain 1 of CD4 were detected in the upper third of vaginal and ectocervical epithelium in four to six out of six specimens examined, using mAb ADP318 or ADP302 (Table 3).

A comparison of RFD1$^+$ dendritic cells with CD1$^+$ Langerhans' cells

Whereas Langerhans' cells were not found in the urethra of adult macaques, human fetuses or human adult rectal epithelium (as reported previously),[38] these tissues showed dendritic-like cell morphology with the RFD1 mAb in the lamina propria of six of eight urethral and five of six rectal specimens (Table 4). This was in contrast to foreskin, vaginal and ectocervical epithelia, all of which had large numbers of Langerhans' cells, but RFD1-staining cells in the lamina

Table 5. Immunohistological findings in human oral, foreskin and macaque urethra specimens; the results are presented as the number of specimens reacting with the given mAb

			Human oral mucosa (n = 16)			Human foreskin (n = 10)			Macaque urethra† (n = 8)		
mAb	Cluster designation	Isotype	Epithelium	Langerhans' cells	Lamina propria	Epithelium	Langerhans' cells	Lamina propria	Epithelium	Langerhans' cells	Lamina propria
HLA-DR		IgG2a	0*	16	16	0*	10	10	3	0	8
HLA-DR‡		IgM	0*	16	16	0*	10	10	NT	NT	NT
GM11		IgG	NC	NC	NC	NC	NC	NC	3	0	8
FcγR1	CD64	IgG1	0	2	6	5	0	5	0	0	0
FcγRII	CD32	IgG2a	0	3	10	0	0	4	5	0	8
FcγRIII											
Leu-11b	CD16	IgM	5§	0	8	7	0	4	3	0	8
3G8	CD16	IgG1	5§	0	8	7	0	4	3	0	8
Leu-2a	CD8	IgG1	NT	NT	NT	0	0	10	0*	0	8
Macrophages	CD68	IgG	NT	NT	NT	0	0	10	0*	0	8
IF-5 (B cell)	CD20	IgG	NT	NT	NT	0	0	10	0	0	0
CR2 (B cell)	CD21	IgG2a	0	0	0	0	0	0	0	0	2

* Mononuclear cells infiltrating the epithelium.
† Human fetal urethra expressed FcγRII in five of seven and FcγRIII in one of seven specimens.
‡ RFDR1 (Royal Free Hospital).
§ Only gingival epithelium expressed FcγRIII in five of five specimens.
NT, not tested.
NC, not cross-reacting simian MHC class II antibody.

propria were seen only in two of 10 and one of seven specimens (Table 4). The possibility that the RFD1$^+$ cells in the urethra and rectum might belong to a B-cell subset cannot be excluded, but the morphology of these cells appeared dendritic and B-cell staining with the CD20 or CD21 mAb was not detected.

Fcγ receptors in the oral, foreskin and urethral mucosa

FcγIII receptors were detected by mAb CD16 in all specimens of human and macaque gingival epithelium, using the IgM isotype (Leu-11b) and the IgG isotype (3G8) (Table 5; Fig. 3f). However, buccal, labial, lingual or palatal epithelium did not express FcγRIII. FcγI and FcγII receptors were not detected in any oral epithelium examined, using mAb CD64 and CD32, respectively. A small number of Langerhans' cells expressing FcγI and FcγII receptors was detected in the oral epithelium. In contrast, many mononuclear cells expressing FcγI and FcγII receptors were found in the lamina propria and some of them infiltrated the epithelium. As reported previously, FcγII and FcγIII receptors were detected in human rectal and endocervical but not in vaginal epithelium.[35,36]

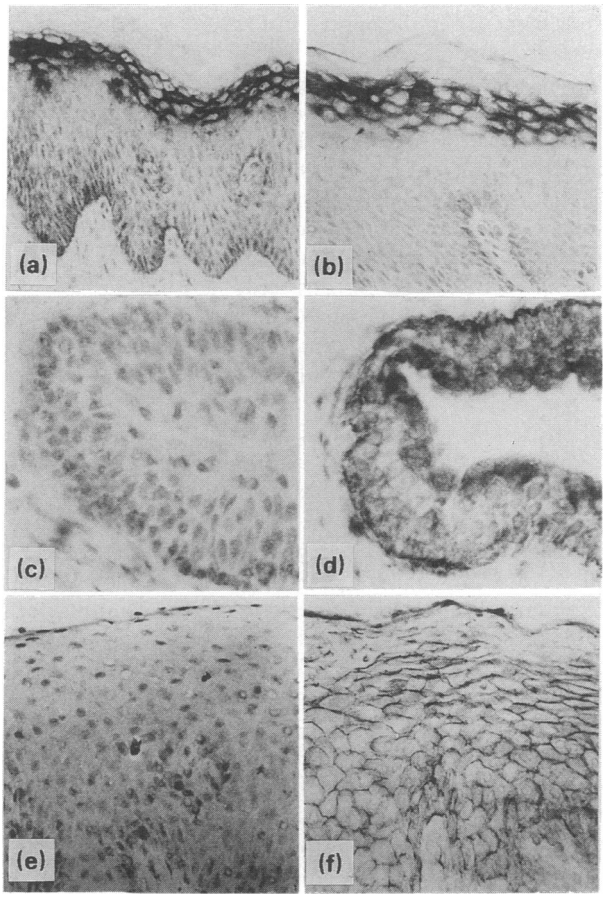

Figure 3. Comparison of FcγI and FcγIII receptors in foreskin, urethral and gingival epithelia. (a) foreskin epithelium, FcγRI (×165); (b) foreskin epithelium, FcγRIII (×330); (c) urethral epithelium (rhesus monkey), FcγRI (×330); (d) urethral epithelium (rhesus monkey), FcγRIII (×330); (e) gingival epithelium, FcγRI (×330); (f) gingival epithelium, FcγRIII (×330).

In the foreskin epithelium, FcγI and FcγIII receptors were detected in five and seven out of 10 specimens, using CD64 and CD16 (Leu-11b, 3G8) mAb, respectively (Fig. 3a and b). FcγRII was not detected in foreskin epithelium. Mononuclear cells expressing FcγRI, FcγRII and FcγRIII were found in the lamina propria (Table 5).

FcγIII receptors were detected in urethral epithelium in three out of eight adult macaques (Fig. 3d) but in only one out of seven human fetal epithelia. FcγII receptors were detected in five out of eight adult macaques (Table 5), but only in a few cells of urethral epithelium of human fetuses. Mononuclear cells expressing FcγRIII and FcγRII were found in the lamina propria of adult macaque urethral specimens. FcγR1 failed to react with any urethral specimens.

HLA-DR in oral, foreskin, urethral and rectal mucosa

HLA-DR was not found in the oral epithelium but Langerhans' cells expressed HLA class II antigen in all specimens examined and were not confined to any part of the epithelium (Table 5). Similarly, HLA-DP and DQ were not detected in the epithelium but all Langerhans' cells reacted with mAb to HLA-DP and four out of 10 with mAb to DQ. In the lamina propria, HLA-DR and, to a lesser extent, DP and DQ were found in the mononuclear cells. In the macaque tissue, Langerhans' cells expressed HLA-DR in all 14 specimens examined, but DP and DQ were not studied.

HLA class II antigen was not detected in the epithelium of the foreskin but HLA-DR was expressed in Langerhans' cells of all specimens examined. HLA-DR was also detected in the mononuclear cells in the lamina propria of the foreskin.

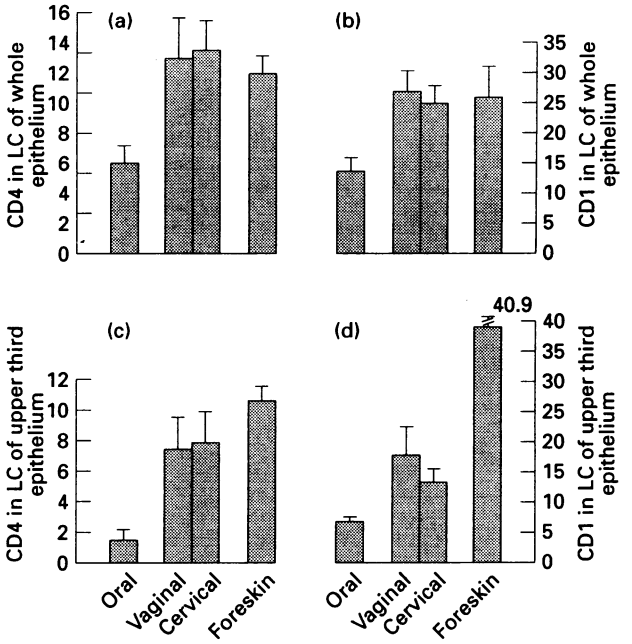

Figure 4. Comparative quantitative assessment of the number of Langerhans' cells (LC) in four epithelia; expressed as mean number of cells (+ SEM) per mm^2.

Urethral epithelial cells in three out of eight adult macaques expressed HLA-DR, using GM11 and HLA-DR mAb (Table 5). HLA-DR was also detected in mononuclear cells in the lamina propria of all eight urethral specimens examined. In addition, some of the DR^+ mononuclear cells were also found in the urethral epithelium in five out of eight macaque specimens. However, HLA-DR^+ cells were found in the lamina propria of only three out of seven human fetal urethral tissues and none in the epithelium. HLA class II antigens (DR, DP and DQ) were also not detected in epithelium cells using four specific mAb. These mAb, however, reacted with mononuclear cells in the lamina propria of all 18 rectal specimens examined (except for HLA-DQ in 13 of 18 specimens).

DISCUSSION

It has been generally assumed that rectal transmission of HIV is facilitated by the thin columnar epithelium, compared with the thick stratified squamous epithelium of vaginal or oral mucosa. In order to establish this quantitatively, we have measured the thickness of these epithelia by means of video image analysis. Indeed, the mean thickness ($\pm$ SD) of oral ($263 \pm 1{\cdot}6\,\mu m$) or vaginal epithelium ($215{\cdot}5 \pm 89{\cdot}2\,\mu m$) is about nine to 12 times greater than that of rectal epithelium ($24{\cdot}6 \pm 9{\cdot}7\,\mu m$). These results are consistent with the view that rectal mucosa is more vulnerable to breaching during rectal intercourse than vaginal or oral epithelium during vaginal or oral sex. This is compounded by the trauma inflicted to the rectal mucosa during intercourse, and the rich vascular bed of the rectal mucosa which may facilitate infected seminal fluid to enter the traumatized blood vessels. Quite apart from the purely morphological features, there is evidence that recurrent infections of the rectum or the cervix and the high incidence of cervical erosions, especially in high-risk groups, facilitate rectal or cervicovaginal transmission of HIV,[4,39] possibly by providing a breach in mucosal integrity. The male foreskin and urethra might also become infected on exposure to HIV-infected cervical secretions during heterosexual intercourse or on direct contact with infected rectal tissues during homosexual intercourse.[2,5–9] The target cells for HIV infection of the male genitourinary tract are unclear, but the presence of foreskin in homosexual men is associated with an increased risk of HIV infection.[9]

For HIV transmission to occur through an intact mucosal surface, the virus must initially bind to a surface receptor on the epithelial cells. In this investigation we have considered three potential receptors, which may enable HIV to bind to the foreskin, urethra or oral epithelium. CD4 glycoprotein binds directly gp120 on the surface of HIV.[21,22] Fcγ receptors may bind cell-free HIV–IgG antibody complexes, and HLA class II antigen might bind $CD4^+$ cell-associated HIV in infected seminal fluid.[40] CD4 glycoprotein has not been detected in foreskin, urethral, oral, rectal[36] or cervicovaginal[35] epithelia, but a few $CD4^+$ endocervical epithelial cells have been found.[35] A small number of $CD4^+$ mononuclear cells have been found to infiltrate the foreskin, urethral, oral, rectal and cervicovaginal epithelia. An abundance of $CD4^+$ mononuclear cells is found in the lamina propria of all these epithelia. Macrophages and $CD8^+$ mononuclear cells were found in the lamina propria of the urethra and foreskin, and in the latter a number of $CD8^+$ cells infiltrated the epithelium. This is of interest, as intra-epithelial lymphocytes (mostly $CD8^+$ cells) and macrophages have been reported in human epididymis[41] and in the epithelium of the male genital tract.[42]

Langerhans' cells were found in the foreskin epithelium and in almost all specimens of oral epithelium, as reported previously,[38,43] but were not detected in the urethral epithelium. However, the number of $CD1^+$ ($P < 0{\cdot}02$) and $CD4^+$ ($P < 0{\cdot}05$) Langerhans' cells was significantly lower in oral than vaginal or ectocervical epithelium. Furthermore, assuming that Langerhans' cells at or near the epithelial surface are most vulnerable to infection with HIV, we found only $1{\cdot}4 \pm 0{\cdot}7$ Langerhans' cells/mm^2 in the upper third of the oral epithelia, compared with $7{\cdot}4 \pm 2{\cdot}2$ and $7{\cdot}8 \pm 2{\cdot}1$ Langerhans' cells in the vaginal and ectocervical epithelia, respectively. Furthermore, the number of $CD4^+$ Langerhans' cells in the upper third of the foreskin epithelium (7–36 year old) was significantly higher ($10{\cdot}6 \pm 0{\cdot}8$ cells/mm^2) than in the oral and cervicovaginal epithelia ($P < 0{\cdot}05$). These results were strengthened by the finding that the gp120 V1-binding domain of CD4 in Langerhans' cells was found in only one out of seven oral epithelial specimens, compared with eight out of 10 specimens in the foreskin epithelium and four to five out of six specimens in vaginal and ectocervical epithelium. However, foreskin from infants had a higher number of $CD1^+$ Langerhans' cells expressing CD4 in the upper third ($23{\cdot}2 \pm 4{\cdot}7$ cells/mm^2) than the corresponding counts found in the older age groups ($10{\cdot}6 \pm 0{\cdot}8$ cells/mm^2). This suggests a decrease of Langerhans' cells with age, but there was an inadequate number of adult foreskin specimens to establish this. However, there is no evidence that the number of Langerhans' cells changes with age either in skin not exposed to sunlight[44] or the oral mucosa.[45] These data raise the possibility that the increased risk of uncircumcised men becoming infected with HIV might be associated with the high number of $CD1^+$ Langerhans' cells near the surface of the foreskin epithelium expressing CD4. In contrast, the paucity of $CD4^+$ Langerhans' cells expressing the V1 domain in oral epithelium argues against oral transmission of HIV.

A surprising finding was that epithelia with Langerhans' cells (foreskin, vagina and ectocervix) showed few $RFD1^+$ dendritic-like cells in the lamina propria. In contrast, urethral and rectal epithelia, with no demonstrable Langerhans' cells showed $RFD1^+$ cells in the lamina propria of almost all specimens examined. Excluding the possibility that the $RFD1^+$ cells might represent a subset of B cells, the results suggest that the absence of epithelial Langerhans' cells is compensated for by the presence of dendritic cells in the lamina propria.

An alternative mechanism for cell-free HIV infection is that of IgG antibody-mediated enhancement of HIV–antibody complexes via Fcγ receptors.[46,47] Indeed, infected seminal fluid contains HIV and IgG antibodies,[48] so immune complexes can be formed. FcγII and FcγIII receptors were detected in urethral, rectal and endocervical [35,36] epithelia, and FcγIII and FcγI receptors in foreskin epithelium, which may enable HIV–antibody complexes to bind to these epithelial cells. However, FcγI and FcγII receptors were not found in oral epithelium and FcγIII receptors were confined to gingival epithelium, which would diminish the chances of oral HIV infection by means of HIV–antibody complexes.

We studied the expression of HLA-DR in urethra, foreskin and oral epithelial tissues, as HIV in seminal fluid might be CD4 cell-associated, and CD4 glycoprotein binds HLA class II

antigen.[49] It is noteworthy that the epithelial cells lining the male genitourinary tract can express HIV class II (DR) antigen,[41] and this may be associated with T-cell activation, especially during infection. HLA class II (DR) was found in three out of eight normal male urethral epithelium of adult non-human primates and in endocervical epithelia of humans.[35] Whether this might enable HLA class II to bind CD4 cell-associated HIV and facilitate infection of these epithelial cells needs to be explored. Normal human and non-human primate oral epithelium, on the other hand, does not normally express HLA class II antigens, so binding of cell-associated HIV is unlikely to occur in the oral cavity.

The comparative investigation of potential HIV binding receptors suggests that: (1) direct infection of $CD4^+$ Langerhans' cells in the foreskin or vagina may be transmitted by the lymphatics to the regional lymph nodes; (2) the presence of Fcγ receptors in epithelial cells of the foreskin, male urethra, rectum and endocervix enables these epithelial cells to bind HIV–antibody complexes; (3) oral epithelial cells generally lack Langerhans' cells expressing CD4 near the epithelial surface to bind to HIV, lack FcγI and II receptors to bind HIV–IgG antibody complexes, and lack HLA class II antigens to bind CD4 cell-associated HIV; (4) in contrast, foreskin epithelium has the largest number of $CD4^+$ Langerhans' cells and the epithelial cells do express FcγI and FcγIII receptors. These results are consistent with the presence of foreskin being a significant risk factor in HIV infection.[8,9] However, the findings argue against oral mucosa being a conducive site for HIV transmission. This conclusion is consistent with the lack of evidence in favour of transmission of HIV by oral sex[10,11] or by intimate kissing between seropositive subjects (harbouring HIV in saliva) and seronegative subjects.[15] It is also possible that a salivary inhibitor may inactivate HIV and prevent infection.[50] However, we wish to emphasize that some common oral lesions, such as recurrent oral ulcers, gingivitis or periodontal disease, may cause a breakdown in the continuity of epithelium, allowing direct access of HIV to the underlying $CD4^+$ cells or indeed to the rich vascular supply. This may be found with inflamed gingival tissue, which may readily break down and bleed on provocation, such as oral sex or passionate kissing.

ACKNOWLEDGMENTS

We are grateful to Professor G. Jannosy and Dr H. Holmes, from the MRC AIDS Directed Programme Reagent Project, for mAb ADP 302, 318, 3015, 359, Dr Q. Sattentau (Centre d'immunologie de Marseille, Luming, France) for the CD4 mAb 5A8, and Dr E. A. Clark (University of Washington, WA) for mAb CD20 (IF5).

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