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Expression and gene transcript of Fc receptors for IgG, HLA class II antigens and Langerhans cells in human cervico-vaginal epithelium

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SUMMARY

The mechanism of transmission of HIV from the male to the female genital tract or in the reverse order is not clear. CD4 glycoprotein is the receptor for HIV and Langerhans cells and the related dendritic cells could play a role in the initial transmission of HIV. Fc receptors (FcR) for IgG might be involved in antibody-mediated binding of HIV. We carried out an immunohistological study of normal human cervical and vaginal epithelia for the presence of CD4 glycoprotein, Langerhans cells and FcR to IgG. $CD4^+$ glycoprotein was not found in the vaginal or cervical epithelium, with the exception of a few endocervical epithelial cells. A small number of $CD4^+$ mononuclear cells were found in the endocervical epithelium of a third of the specimens but a large number of $CD4^+$ cells was found in the submucosa of most of the cervical and vaginal specimens. Langerhans cells expressing CD4, HLA class II, FcγR2 and FcγR3 were detected in most vaginal, ectocervical and transformation zone epithelia and in 9/14 endocervical tissues. FcγR3 was detected in about two-thirds of the columnar endocervical epithelium and the transformation zone. A smaller number of specimens expressed FcγR2 in these epithelia, but FcγR1 was not detected. We then demonstrated mRNA for FcγR3 in the columnar endocervical epithelial cells and transformation zone by *in situ* hybridization, using a CD16-RNA probe. FcγR3 and FcγR2 gene transcripts were also found in fetal cervical tissue by applying the polymerase chain reaction to amplify portions of the FcγR3 and FcγR2 coding sequences in cDNA prepared from fetal RNA. HLA-DR was found in the endocervical cells, transformation zone and in Langerhans cells of all specimens. The presence of Langerhans cells, Fcγ receptors and HLA class II antigen offers three potential mechanisms for cervico-vaginal HIV transmission: (i) direct HIV infection of Langerhans cells, (ii) binding of HIV antibody complexes to cervical epithelial Fcγ receptors and (iii) binding of HIV infected $CD4^+$ cells to cervical HLA class II antigen which may infect these or the adjacent $CD4^+$ cells.

Keywords FcγR Langerhans cells HIV cervico-vaginal mucosa HLA class II

INTRODUCTION

Human immunodeficiency virus (HIV) is the etiological agent of acquired immunodeficiency syndrome (AIDS) [1–3]. In North America, Western Europe and Britain HIV has been most commonly spread by male homosexual activity [4,5] and by contact with HIV-infected blood, through intravenous drug abuse and blood transfusions. However, heterosexual spread appears to be the major route of HIV transmission in Africa [6,7] and it is rapidly increasing in North America, Europe and Britain [8]. HIV has been isolated from the cervico-vaginal secretions of HIV-infected women [9,10], and HIV antigen has been detected in lymphocytes from the cervico-vaginal secretions of seropositive women [11]. HIV has also been detected by immunohistology and electron microscopy in dendritic cells and Langerhans cells of patients with AIDS [12,13]. Recently, SIV was found to infect Langerhans cells and macrophages of the genital tract epithelium of rhesus monkeys [14]. Langerhans cells are bone marrow derived dendritic cells and are the only epidermal cells that exhibit class II antigens, Fcγ and C3 receptors [15], CD1 and to a lesser extent CD4 antigens [16,17].

The main receptor for HIV is the CD4 glycoprotein which is expressed by CD4 lymphocytes and to a lesser extent monocytes, macrophages and Langerhans cells [18,19]. HIV in seminal fluid may bind directly to Langerhans cells in the cervico-vaginal epithelium, the cells can enter the afferent lymphatics and travel to the genital lymph nodes [20]. Alternatively, enhancing IgG antibodies may play a role in HIV infection as Fc receptors to IgG can bind HIV antibodies and enhance HIV infections in human monocyte cell lines [21,22], macrophages and $CD4^+$ lymphocytes [23,24] and in non-

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haematopoietic cells with induced FcγR [25]. This may either enable the cervico-vaginal epithelium to become permissive to HIV infection or the complexes might move to the basal epithelial cell surface, where they can bind directly to CD4 cells in the sub-epithelial stroma. Indeed, we have postulated that Fcγ receptors on rectal epithelial cells may facilitate binding of virus-antibody complexes [26].

The objectives of this investigation were to study the distribution of Langerhans cells and to find out if CD4 glycoprotein or Fc receptors for IgG are detectable in the cervico-vaginal epithelium. It is evident that at least two mechanisms are available for cervico-vaginal HIV transmission: direct HIV infection of Langerhans or dendritic cells or binding of HIV-antibody complexes to putative Fcγ receptors on epithelial cells which may infect these or the adjacent $CD4^+$ cells. Indeed, we detected, by immunohistology, FcγR3 and FcγR2 in clinically normal endocervical and transformation zone epithelial cells. Langerhans cells were also found in these and in vaginal epithelial cells. FcγR3 mRNA was then demonstrated by *in situ* hybridization with a CD16-RNA probe. The presence of FcγR3 and FcγR2 gene transcripts was also demonstrated by using polymerase chain reaction to amplify portions of FcγR3 and FcγR2 coding sequences in cDNA prepared from the fetal RNA. Furthermore, in order to identify potential binding molecules for cell-bound HIV in $CD4^+$ cells of seminal fluid, we studied HLA class II expression in cervico-vaginal epithelial cells and detected predominantly HLA-DR in the endocervical and transformation zone cells.

MATERIALS AND METHODS

Collection and preparation of tissue sections

Normal cervico-vaginal tissue was obtained from 14 patients undergoing hysterectomy in which no macroscopic cervical abnormalities were present. The patients were between 34 and 52 years of age, 12 of whom were pre-menopausal and two were post-menopausal. In all cases a block of tissue was removed to include the ectocervical epithelium, transformation zone and endocervical epithelium. In addition 10 specimens of normal vaginal tissue were included in this study. Fetal cervico-vaginal tissue was removed from six human fetuses, at 17–22 weeks of age and stored in liquid nitrogen. The tissue was embedded in OCT compound (Miles-Elkhart, IN), frozen in liquid nitrogen and then stored at −70°C. Cryostat sections (6 μm thick) were cut and placed on gelatine-coated glass slides, and air dried for 1 h. Sections of all samples were stained with haematoxylin and eosin for histological examination.

Monoclonal antibodies

A series of MoAb was used in this study against CD4 glycoprotein, Langerhans cells, HLA DR, DP and DQ and Fc receptors (FcR1, FcR2, FcR3) to IgG. The antibody class, molecular weight, source and tissue specificities are given in Table 1.

Immunohistology

The indirect immunoperoxidase technique was applied using the avidin-biotin-peroxidase complex method [27]. The sections were fixed in acetone for 10 min, then incubated with optimal dilution of MoAb for 75 min. Normal rabbit or goat serum was used to block non-specific binding, followed by incubation with 1:200 dilution of biotinylated rabbit anti-mouse IgG $F(ab)_2$ (Serotec, Kidlington, Oxford, UK) or biotinylated goat anti-mouse IgM $(F(ab)_2$ (Tago, Burlingame, CA) for 1 h, followed by streptavidin horseradish peroxidase complex (Dakopatts, Glostrup, Denmark) for 30 min at room temperature, in a moist chamber. Each incubation was followed by washing in two changes of Tris-buffered saline (pH 7·6). The sections were then

Table 1. Monoclonal antibodies used in this study

Name	Cluster designation	Clone no.	Class	Molecular weight of antigen (kD)	Optimal dilution	Source	Specificity in normal tissues
Leu 3a	CD4	SK3	IgG1	55	1:10	Becton Dickinson	Helper/inducer T cell
FcγR1 22	CD64		IgG1	70	1:30	Medarex	Monocytes, macrophages
32.2					1:30	Medarex	Monocytes, macrophages
FcγR2	CD32	2E1	IgG2a	48	1:10	Immunotech	Granulocytes, monocytes platelets and B lymphocytes
FcγR3(Leu 11b)	CD16	GO22	IgM	50–70	Neat	Becton Dickinson	Neutrophil-NK cells-epithelial cells peripheral blood lymphocytes
HTA1-C1	CD1	NA1/34 HLK	IgG2	49	1:20	Sera-lab	Langerhans cells in the skin and rare dendritic cells in the lymph nodes and thymus
RFDR1			IgM	28/33	1:5	Dr L. Poulter 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
NKHIA		3B8	IgM	200	1:30	Coulter Immunology	Large granular lymphocytes NK cells

All MoAb were purified immunoglobulins, except RFDR1 and HLA-DQ which were tissue culture supernatants.

stained by incubating them in a solution of 3,3-diaminobenzidine (DAB) tetrahydrochloride (Sigma, Poole, UK) and hydrogen peroxide for 10 min [28]. After washing, the sections were counterstained with haematoxylin, dehydrated in graded alcohol, cleared in xylene and mounted in DPX. The optimal dilution for each MoAb was determined on sections of tonsils which were also used as positive controls. Non-specific reaction with the second layer antibody was controlled for each MoAb by omitting the latter. Isotype specificity of CD16 (IgM) for FcγR3 was tested by using corresponding IgM isotype of unrelated MoAb (NKHIA, Table 1) which consistently failed to react with epithelial cells. The immunoperoxidase preparations were examined independently by two of the authors (L.H. and T.L.), using a standard Zeiss microscope and Leitz Orthoplan microscope, respectively.

Immunohistology and morphometric analysis of Langerhans cells

Cell counts were performed with the Zeiss MOP-Videoplan image analysis system (64K Z80 microprocessor Kontron), attached to Zeiss microscope with a digitizing tablet. An eyepiece graticule was fitted to the microscope. Stained sections were first scanned using a ×40 objective and four or five areas of the epithelium were selected at about equal intervals along the entire length of each specimen to count CD1-stained Langerhans cells identified by a definable cell body. The area of epithelium within which the cells were counted was then measured by using a light cursor to trace the outline of the area of the epithelium in order to calculate the number of CD1-labelled cells per given epithelial area. The results were expressed as the number of Langerhans cells/mm^2.

In situ *hybridization*

Cryostat sections (6 μm) of cervical biopsies were cut, fixed onto 3-aminopropyltriethoxysilane (Sigma) coated microscope slides with 4% paraformaldehyde for 25 min and washed twice in PBS. Slides were dehydrated in graded alcohols and stored dry at −70°C. Slides were thawed and washed in 0·1% Triton X-100 (Sigma) in 2 × SSC (SSC is 0·15 M NaCl, 0·015 M Na citrate) for 10 min at 22°C before permeabilization with proteinase K (Sigma), 0·5 μg/ml, for 10 min at 37°C. The sections were rinsed in glycine, 2 mg/ml in 100 mM Tris HCl pH 7·8, before post-fixation in 4% paraformaldehyde. Slides were subsequently treated with 0·25% acetic anhydride (Sigma) for 10 min at 22°C and washed twice in 2 × SSC before dehydration through graded alcohols. The sections were pre-hybridized with 0·25 mg/ml sheared salmon sperm DNA in 25% formamide, 0·5 × Denhardt's solution, 5% dextran sulphate, 50 mM dithiothreitol (DTT; Sigma), in 2 × SSPE (0·3 M NaCl, 2 mM EDTA in 0·02 M NaH_2PO_4, pH 7·4) for 1 h, at 42°C and then washed twice in 2 × SSC at room temperature. Sections were then dehydrated in alcohol.

RNA probes (typically 3×10^5 ct/min) labelled as described below were resuspended in 50% formamide, 1 × Denhardt's solution, 0·5 mg/ml sheared salmon sperm DNA, 10% dextran sulphate and 100 mM DTT in 2 × SSPE and applied to the sections. Incubation was for 16 h at 42°C. The slides were washed in 2 × SSC followed by 10 mM DTT in 0·1 × SSC at 37°C. After dehydration, slides were dipped in D-19 emulsion (Kodak, Hemel Hempstead) in 5% glycerol and autoradiographed for 3–5 days. Sections were counterstained with haematoxylin.

CD16 cDNA from the plasmid pCD16 [29] was subcloned into the transcriptional vector pT3/T7 (Clontech Laboratories Inc., Palo Alto, CA). The orientation of the insert was established by digestion with restriction endonucleases and confirmed by the use of RNA probes in Northern blots. RNA probes incoporating [^{35}S] UTPαS were synthesized using T3 and T7 RNA polymerases with linearized DNA template as described in the Stratagene handbook. Incorporation of radioisotope was determined on aliquots applied to DE81 filters. Probes were partially hydrolysed by treatment with 0·25 M Na_2CO_3 (60°C for 1 h) and applied to tissue sections as above.

Amplification of FcγR3 and FcγR2 gene transcripts

The cDNA-polymerase chain reaction (PCR) was used to amplify fragments from total cellular RNA, corresponding to the coding sequences at the 3′ end of FcγR3 and FcγR2. Total cellular RNA was prepared from fetal tissues by the acid guanidine isothiocyanate method, and 1 μg samples were used to prepare cDNA with reverse transcriptase. Fragments of cDNA were amplified by PCR using the oligonucleotide primers shown in Table 2. The use of these primers has been described previously [26]. Primers 1 and 2 were used to amplify FcγR3 transcripts, primers 3 and 5 were used for the amplification of FcγR2A transcripts, primers 4 and 5 for FcγR2B and primers 4 and 6 for FcγR2C transcripts. The mismatched primers 3 and 6 were used in control reactions to confirm the specificity of the primers for the FcγR2 isoforms. The PCR was carried out in a reaction volume of 100 μl, using a thermal cycler (Techne Ltd, Cambridge, UK); 30 cycles, at 94°C for 30 s, 55°C for 1 min and 72°C for 1 min. Controls consisted of a reaction mixture in which reverse transcriptase was omitted or in which RNA from a monkey kidney epithelial cell line (LLC-MK2) was used as template. Aliquots of 10 μl were analysed on 2% or 3% (w/v) agarose gels and after transfer to nitrocellulose they were hybridized with randomly labelled probes of CD16 (for FcγR3) or an end-labelled oligonucleotide probe (oligonucleotide 7, Table 2) common to all FcγR2 isoforms.

FcγR3 isoform expression

Amplified FcγR3 fragments were analysed by Southern blot to determine whether they were derived from gene transcripts of the NK (FcγR3A) or neutrophil (FcγR3B) form [30]. Oligonucleotide probes (No. 8 and 9 in Table 2) were those described previously [30]. FcγR3 fragments amplified from polymorphonuclear leucocytes (PMN) or an NK-cell-enriched population were used as controls. PMN were prepared by centrifugation of peripheral blood on Polymorphoprep (Nycomed, Oslo, Norway) according to the manufacturer's instructions. A fraction enriched for NK cells was prepared from the sheep erythrocyte rosetting population by depletion of CD4 cells by panning.

RESULTS

Immunohistology of CD4$^+$ cells

Sections of the cervix show three types of epithelia; endocervical columnar epithelium, metaplastic squamous epithelium of the transformation zone and ectocervical squamous epithelium. CD4 glycoprotein was not detected with Leu3a MoAb in the vaginal or ectocervical squamous epithelium but was detected in parts of the endocervical columnar epithelium in 2/14 specimens. Furthermore, isolated CD4$^+$ mononuclear cells were

Table 2. Oligonucleotide primers and probes used in polymerase chain reaction (PCR) identification of FcγR3 and FcγR2 transcripts

Oligonucleotide	Sequence	Nucleotides
1. CACTGCTCTGCATAAGGTCAC	FcγR3	399–419
2. CAAATGTTTGTCTTCACAGAG	FcγR3	681–701 (c)
3. GTGCCCAGCATGGGCAGC	FcγR2A	619–636
4. GCTCCCAGCTCTTCACCG	FcγR2B	646–663
	FcγR2C	646–663
5. GTTATTACTGTTGACATGGTC	FcγR2A	932–952 (c)
	FcγR2B	949–969 (c)
6. AATACGGTTCTGGTCATCAGG	FcγR2C	853–873 (c)
7. GCCTTGATCTACTGCAGGAAAAAGCG	FcγR2 A	709–736
	B	727–752
	C	727–752
8. ACAAACATTTGAAGCTCA	FcγR3 (neutrophil)	699–717
9. ACAAACATTCGAAGCTCA	FcγR3 (NK)	699–717

(c) complementary.
Nucleotide positions from cDNA sequences 32,36.

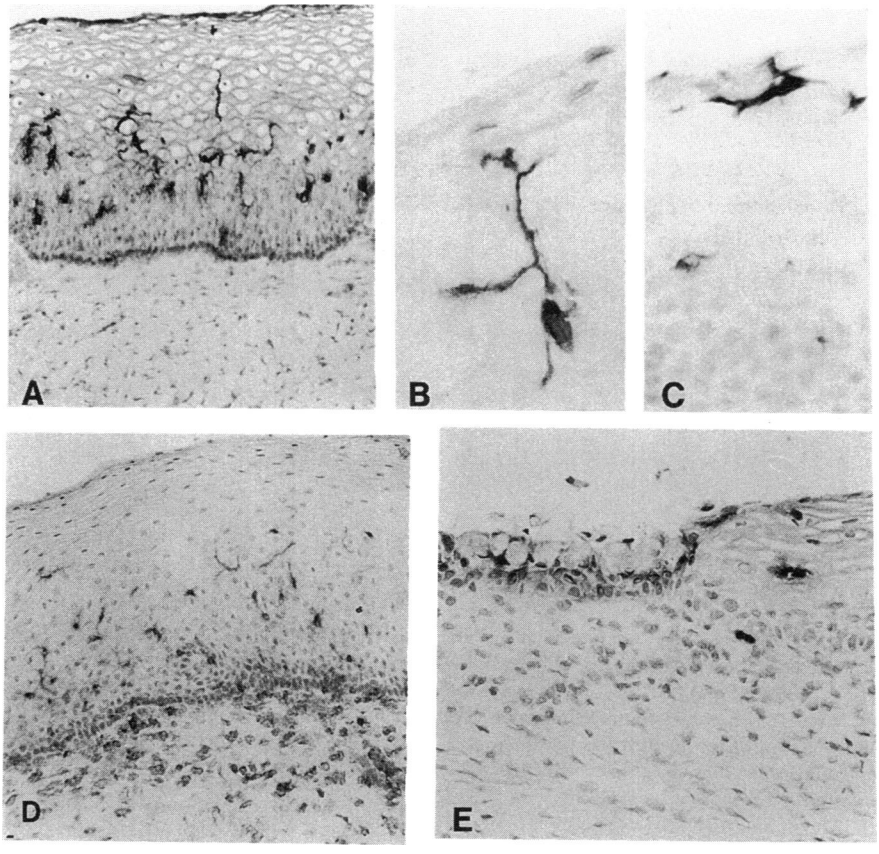

Fig. 1. Immunoperoxidase staining of Langerhans cells in cervico-vaginal epithelium revealed by MoAb CD1 (NA1/34). (A) CD1 Langerhans cells in ectocervical epithelium, × 50. (B) Langerhans cells near the surface of the epithelium, × 200. (C) Langerhans cells cytoplasmic processes extended to the surface of the epithelium, × 200. (D) Langerhans cells expressing CD4 glycoprotein in ectocervical epithelium revealed by MoAb (Leu3a), × 50. (E) Langerhans cells in the transformation zone and endocervical columnar epithelium, × 50.

found in 5/14 specimens of endocervical epithelium. Langerhans cells expressing CD4 glycoprotein were found in vaginal and ectocervical epithelium (Fig. 1D). CD4$^+$ cells were found in 12 out of 14 cervical biopsies in the subepithelial stroma adjacent to columnar endocervical, transformation and squamous epithelia, rather than deep in the cervical connective tissues (Fig. 2F). In addition, many CD4$^+$ cells were found in the basement membrane and some of the cells infiltrated the cervical epithelium. No difference in CD4 expression was found in pre-menopausal and post-menopausal specimens. Examination of

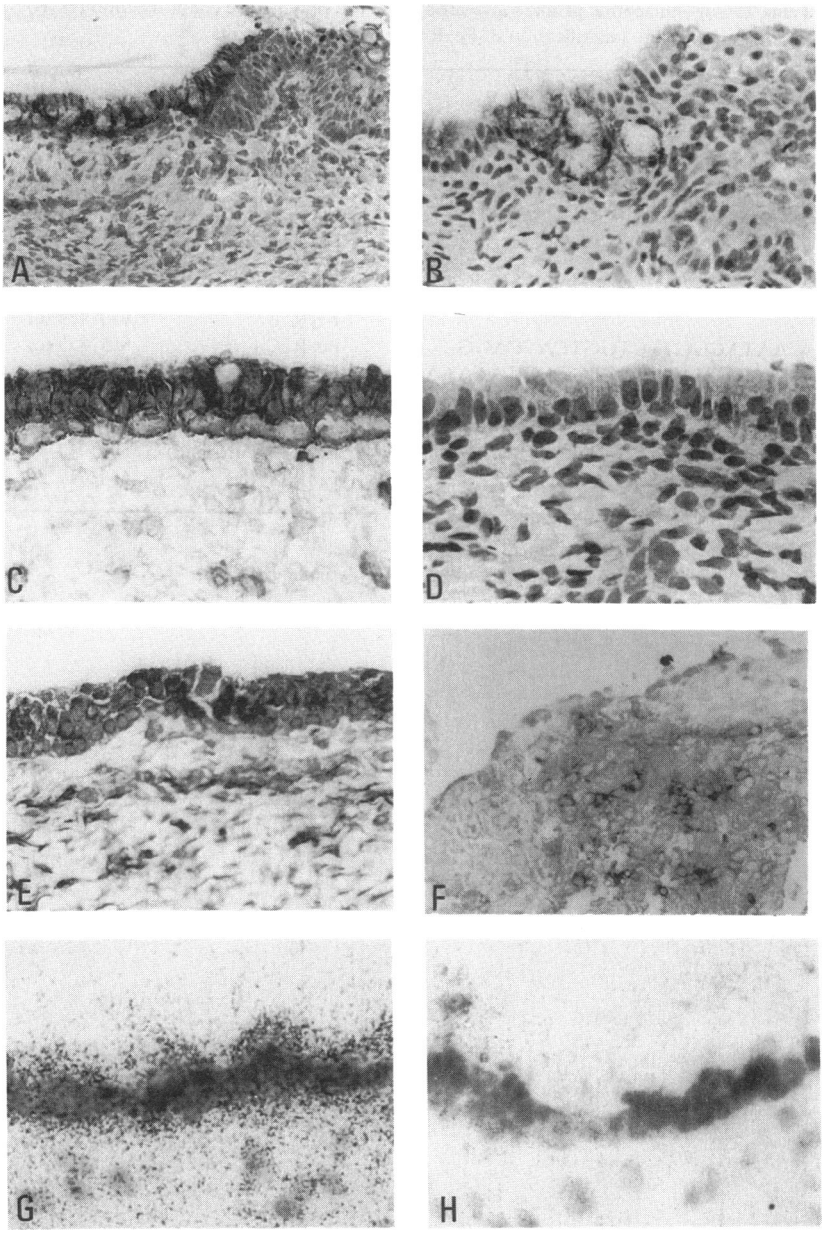

Fig. 2. Immunoperoxidase staining of cervical epithelium and identification of CD16 mRNA by *in situ* hybridization. (A) FcγR3 in transformation zone and endocervical columnar epithelial cells revealed by the IgM class of MoAb (CD16), × 130. (B) No staining by omitting the MoAb, × 130. (C) FcγR3 (CD16) in endocervical columnar epithelium, × 200. (D) Control showing no staining by omitting the MoAb, × 200. (E) HLA-class II antigen in endocervical columnar epithelium revealed by MoAb (HLA-DR), × 200. (F) $CD4^+$ cells in submucosal cervical stroma revealed by MoAb (Leu3a), × 86. (G) The presence of CD16 mRNA in epithelial cells was demonstrated by *in situ* hybridization using ^{35}S-labelled CD16 antisense RNA probe, × 200. (H) No staining was evident following applications of the control (sense) probe, × 200.

Table 3. Langerhans cells count in cervico-vaginal epithelium

	Vagina	Ectocervix	Transformation zone	Endocervix
1. No. of specimens counted	8	8	5	8
2. Mean no. of cells (±s.e.m.) per mm^2	30·1 (±4·3)	40·7 (±4·43)	16·6 (±1·07)	39·6 (±3·3)
3. Range of the no. of cells per mm^2	19–50	33–68	14–20*	33–52

* Counting LC in the transformation zone has been limited in area and based on a smaller sample which affects the accuracy of this count.

Table 4. Immunohistological findings of CD4 glycoprotein, CD1$^+$ Langerhans cells, HLA-class II and FCγ receptors in 14 human cervical and 10 human vaginal specimens; the numbers given are those reacting with each MoAb

			Cervical epithelium ($n=14$)				Vaginal epithelium ($n=10$)	
Monoclonal name	Cluster designation	Isotype	Ectocervix	Transformation zone¶	Endocervical canal	Stroma	Epithelium	Lamina propria
Leu3a	CD4	IgG1	0§	0	2	12	0§	10
NA1/34	CD1	IgG2	14	6/7	9	14	10	0
HLA-DR*		IgM	0‡	11/11	14†	14	0‡	10
HLA-DR		IgG2a	0‡	10/11	13†	14	0‡	10
HLA-DP		IgG1	0‡	0‡	2	9	0‡	10
HLA-DQ		IgG	0	0	0	0	0	2
FcγR1	CD64	IgG1	0	0	0	7	0	0
FcγR2	CD32	IgG2a	0	2/11	5	5	0	0
FcγR3	CD16	IgM	0	7/11	10	10	0	0

* RFDR1 (Royal Free Hospital).
† The cervical glandular epithelium also reacted with MoAb to HLA-DR in eight specimens.
‡ Langerhans cells and mononuclear infiltrating cells were, however, detected with MoAb to HLA DR and DP.
§ In addition Langerhans cells and mononuclear infiltrating cells stained with MoAb to CD4 in vaginal and ectocervical epithelia.
¶ Only 11 or 7 samples were examined with an intact transformation zone.

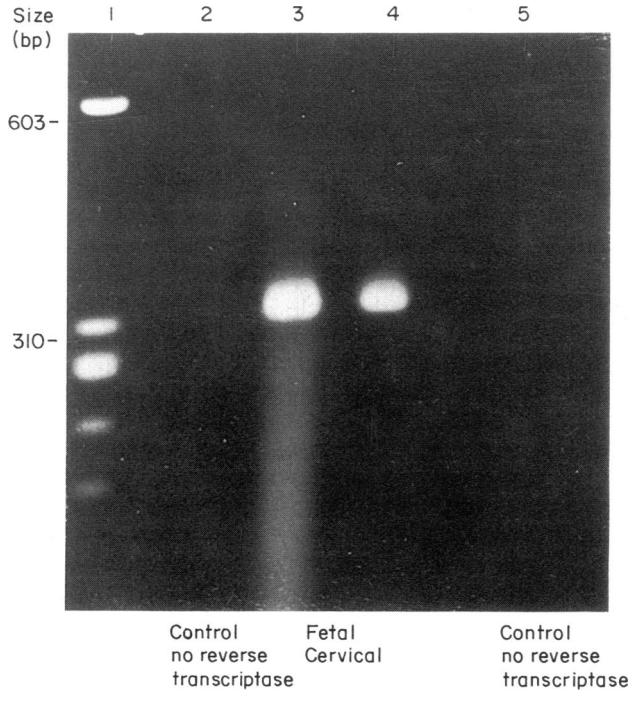

Fig. 3. Identification of FcγR3 transcripts in fetal cervico-vaginal tissue. Aliquots from the cDNA-PCR reaction were analysed by agarose gel electrophoresis and stained with ethidium bromide. Lanes 3 and 4 show the amplification products from two fetal specimens and lanes 2 and 5 are controls where reverse transcriptase was omitted from the reaction. The size marker (lane 1) is ϕx 174 DNA digested with *Hae*III.

the vaginal biopsies also showed CD4$^+$ cells in the lamina propria adjacent to the epithelium, as well as deep in the connective tissue in all 10 biopsies examined. Intra-epithelial CD4$^+$ T cells were also found in all sections.

Immunohistology of Langerhans cells

Langerhans cells were detected in the cervico-vaginal epithelium using CD1 specific MoAb (NA1/34). Large numbers of Langerhans cells were found in the ectocervical epithelium; they showed a body and one to three cytoplasmic processes which extended between adjacent squamous epithelial cells (Fig. 1A). The cells were localized in the basal and suprabasal part of the epithelium but a number of Langerhans cells were also detected near the surface of the epithelium (Fig. 1B) and some of their cytoplasmic processes extended to the surface of the epithelium (Fig. 1C). It seems that the basal Langerhans cells have fewer cytoplasmic processes than those present in the rest of the epithelium but the processes can extend across the basement membrane. The number of Langerhans cells in the ectocervix varied between 33 and 68 cells/mm^2 and the mean number was 40·7($\pm$4·43) (Table 3). CD1$^+$ Langerhans cells were also detected in 6/7 transformation zones (Fig. 1E) and the number varied between 14 and 20 cells/mm^2. The CD1$^+$ Langerhans cells in endocervical epithelium were detected in 9/14 specimens (Fig. 1E) and the number varied between 33 and 52 cells/mm^2. In the vaginal epithelium most of the CD1$^+$ Langerhans cells were localized in the basal and suprabasal epithelium and fewer cells were present than in the cervix; the mean number was 30·1($\pm$4·3) and the number varied between 19 and 50 cells/mm^2 (Table 3). Most Langerhans cells expressed CD4 (Leu3a) and HLA DR and a small proportion of these cells expressed FcγR3 and FcγR2. CD1$^+$ Langerhans cells were also detected in the cervical stroma, adjacent to the epithelium and in the columnar epithelium of the cervical cysts.

Immunohistology of HLA class 2 antigens

Endocervical epithelial cells and transformation zone but not the ectocervical epithelial cells reacted strongly with MoAb (RFDR1) to the HLA-DR antigen in all the specimens examined (Fig. 2E) and all but one specimen reacted with another MoAb to HLA-DR (Table 4). It is noteworthy that the HLA-DR positive cells in the transformation zone and endocervical epithelia were also positive for FcγR3. Endocervical gland epithelium also reacted with the MoAb to HLA-DR in 8/14 specimens. However, squamous epithelial cells of the ectocervix and vagina failed to react with these MoAb, although Langerhans cells which express HLA-DR antigen were readily identified in the basal and suprabasal zones of these epithelia. HLA-DR was also detected in monocytes, lymphocytes and endothelial cells lining the blood vessels in the cervical stroma and in the vaginal lamina propria of all specimens. Of the other HLA class II molecules examined, HLA-DP was expressed by columnar epithelial cells in only 2/14 cervical biopsies and HLA-DQ was not detected. In vaginal tissue, most of the cells stained with MoAb to HLA-DP in the epithelium were Langerhans cells and these were found in 6/10 specimens. Mononuclear cells in the stroma reacted with the MoAb to HLA-DP in 9/14 cervical and 10/10 vaginal specimens.

Immunohistology of Fcγ receptors

Fcγ3 receptors were detected by MoAb CD16 in the squamo-columnar junction of the transformation zone in 7/11 and in the columnar endocervical epithelium in 10/14 specimens (Fig. 2A). FcγR2 was detected in the endocervical epithelium in 5/14 specimens with MoAb CD32, and in 2/11 in the transformation zone but FcγR1 was not found with MoAb CD64. Ectocervical and vaginal epithelia failed to react with any of the MoAb to FcγR. Mononuclear cells beneath all the cervical epithelia reacted with MoAb to FcγR3 (10/14), FcγR2 (5/14) and also FcγR1 in (7/14) specimens. It is significant that in eight out of 10 FcγR3 positive endocervical epithelia, $CD4^+$ cells which stained with Leu3a were detected in the subepithelial stroma. Of these epithelia four also expressed FcγR2. However, four specimens showed $CD4^+$ cells in the stroma without any Fcγ receptors expressed in the epithelial cells. A small number of cells which resemble Langerhans cells in the cervical squamous epithelium stained with MoAb to CD16 and CD32 antigen. Routine controls included omission of IgM (Fig. 2D) or IgG MoAb before addition of the biotinylated second layer antiserum and these were consistently negative. Isotype specificity of CD16 (IgM) for FcγR3 was tested by using the corresponding IgM isotype of an unrelated MoAb (NKHIA) which failed to react with epithelial cells.

In situ *hybridization for FcγR3 mRNA*

The presence of CD16 mRNA transcripts in biopsy sections was then examined by *in situ* hybridization. CD16 mRNA was detected in the transformation zone and endocervical but not in the ectocervical or vaginal epithelium (Fig. 2G). The localization pattern of mRNA by *in situ* hybridization was similar to that found by staining with MoAb specific for the CD16 surface protein (FcγR3). No significant hybridization was observed with control (sense) RNA probe prepared with the T7 RNA polymerase (Fig. 2H).

Amplification of FcγR3 and FcγR2 gene transcripts in fetal tissue

FcγR2 and FcγR3 may be passively acquired on the cell surface following their release from stimulated neutrophils [31]. Furthermore, these receptors can be induced by viral infections [25]. In order to establish that FcγR3 and FcγR2 are expressed constitutively, we examined fetal tissue in which active or passive acquisition of these receptors is unlikely to have occurred. We prepared cDNA from total cytoplasmic RNA and then amplified a portion of the FcγR3 and FcγR2 coding sequence by the polymerase chain reaction. For FcγR3 a fragment of the expected size (308 bp) was amplified using template RNA from two fetal cervical specimens (Fig. 3). No amplification of this fragment was observed in the absence of reverse transcriptase. Since sequences present in different exons were chosen for the PCR primers, the presence of any DNA in the RNA preparations would not give rise to the 308 bp product. The identity of the amplified FcγR3 transcript was confirmed by Southern blotting, using the cloned FcγR3 cDNA probe. Two forms of FcγR3 have been described; FcγR3A which is expressed by natural killer cells and macrophages is a transmembrane protein with a cytoplasmic domain [30] whereas FcγR3B is expressed by neutrophils and is anchored by a glycosylphosphatidylinositol linkage [30]. Southern blot analysis of the PCR products from fetal tissue, using oligonucleotide probes specific for each form [30], indicated that they were from FcγR3B gene transcripts (results not shown). Portions of the FcγR2 coding sequences were then amplified and fragments of all three isoforms (FcγR2A, FcγR2B and FcγR2C) were detected by Southern blot analysis, although only the FcγR2A product was visualized on staining with ethidium bromide (results not shown). As reported previously for fetal colorectal tissue [26] the apparent size of the FcγR2B product (approximately 300 bp) was smaller than expected (336 bp).

DISCUSSION

The CD4 glycoprotein is the main receptor for HIV [18,19]. We have not detected CD4 glycoprotein in cervico-vaginal epithelium by immunohistology with MoAb Leu3a, but some endocervical epithelial cells expressed CD4 glycoprotein in 2/14 specimens. $CD4^+$ Langerhans cells and mononuclear cells were found in these epithelial tissues. We have detected $CD1^+$ Langerhans cells in almost all specimens of vaginal and ectocervical squamous epithelia and in the transformation zone of the cervix which may well represent the most vulnerable part of the female genital tract in HIV transmission. These cells were also found in two-thirds of the specimens of endocervical columnar epithelium. Langerhans cells or dendritic cells could play an important role in the initial spread of HIV, as the latter was identified by electron microscopy in some Langerhans cells from AIDS patients [13] and purified Langerhans and dendritic cells can be infected *in vitro* by HIV [32,33]. However, others failed to find HIV in Langerhans cells from HIV-infected patients [34,35]. We suggest that HIV in seminal fluid may infect Langerhans cells directly in the cervico-vaginal epithelium and the cells may pass to the sub-epithelial $CD4^+$ cells or enter the afferent lymphatics and travel to the lymph nodes and activate and infect $CD4^+$ T cells.

It is debatable whether the limited expression of CD4 glycoprotein on the epithelial surface and the controversial role

of Langerhans cells are adequate to account for HIV binding. Hence, alternative means of transmission of HIV from seminal fluid to the female genital tract were considered. Indeed, enhancing antibodies can be involved in HIV infection of human lymphoid [21–24] and non-lymphoid cells [25]. HIV infection of epithelial cells has received limited attention, though infection of HeLa and colorectal carcinoma cell lines has been reported [36–38]. The cell lines susceptible to HIV infection have not been characterized for Fc receptors, but they did not express surface CD4 molecules, though some of the cells contained CD4-RNA [36,37]. Several studies have shown that cell lines become susceptible to HIV infection only after transfection with the CD4 gene [39]. Immunohistological investigation of Fcγ receptors revealed FcγR3 (CD16) and FcγR2 (CD32) were detected exclusively in the endocervical epithelial cells and in the transformation zone, but FcγR1 (CD64) was not found in any epithelial cells (Table 4). The expression of FcγR3 and/or FcγR2 on the surface of epithelial cells in the endocervical canal or transformation zone may enable the HIV-antibody complex to bind to these epithelial Fcγ receptors. This may enable the cervico-vaginal epithelium to become permissive to HIV infection or the complex might move to the basal epithelial cell surface, where it can bind directly to CD4 cells in sub-epithelial stroma and proliferate within these cells [40].

In order to identify potential binding molecules for CD4 cells infected with HIV in seminal fluid, we studied HLA class II expression in cervico-vaginal epithelial cells. HLA-DR was expressed in all cervical specimens examined and was again localized exclusively to the endocervical epithelial cells and transformation zone. HLA-DP was detected only in a few specimens and HLA-DQ was not found in any of the tissues examined (Table 4). There is some evidence that human sperms can be infected with HIV and that sperms can bind to HLA-DR molecules through a structure that is antigenically similar to CD4 [41]. The expression of HLA-DR in the endocervical epithelium and transformation zone may enable CD4 cell or sperm bound HIV to bind and infect epithelial cells.

There is as yet no evidence that HIV–antibody complexes bound to Fcγ receptors on endocervical epithelial cells activate these cells to become permissive for HIV infection. However, antibody mediated attachment of virus to Fcγ receptors on monocytes has been considered to induce infection with dengue virus [42] or yellow fever virus [43]. If these epithelial cells were not to be permissive for HIV, an alternative mechanism is for the FcγR–HIV antibody complexes to be transported from the free to the basal epithelial cell surface, where they may encounter $CD4^+$ lymphocytes or monocytes which are permissive to HIV [40]. It is noteworthy that Fcγ3 and Fcγ2 receptors were also found in human rectal epithelial cells [26] but HLA-class II antigens were not detected in rectal epithelium from healthy heterosexual human subjects.

It is evident that three mechanisms are available for HIV transmission through the cervico-vaginal epithelium: (i) direct HIV infection of Langerhans cells; (ii) binding of HIV-antibody complexes to Fcγ receptors on endocervical epithelial cells; and (iii) binding of HIV-infected CD4 cells or sperms to HLA class II antigen expressed on cervical epithelial cells which may become infected or transmit the HIV to adjacent $CD4^+$ cells. The role of these three modes of mucosal entry of HIV needs to be urgently investigated.

Although the Fcγ receptors expressed by the endocervical cells and the transformation zone have been described with reference to HIV infection, they may be relevant to other cervical infections. Antibodies to any of the sexually transmitted disease organisms (human papilloma viruses, herpes simplex, candida, gonococci, chlamydia, spirochetes and trichomonas) will form immune complexes which may bind to the Fcγ receptors on endocervical and transformation zone epithelial cells by means of the Fcγ portion of IgG antibody. This may enhance transmission of the microorganisms through the epithelial cells.

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