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Investigation of the complement receptor 3 (CD11b/CD18) in human rectal epithelium

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SUMMARY

Rectal and cervicovaginal mucosa are common routes of transmission of HIV, although the mechanism of transmission is unknown. We have investigated human rectal and cervicovaginal epithelia for the expression of complement receptors (CR) and cell adhesion molecules which may be involved in HIV and other infections. In rectal mucosa, CR3 was detected in the surface and crypt epithelial cells by immunohistology, using MoAbs to CD18 and CD11b in 10 out of 15 specimens. RNA transcripts encoding both CD11b and CD18 were also demonstrated in surface and crypt epithelial cells by *in situ* hybridization. Although CD11b was detected in the epithelial cells in three out of the 14 cervicovaginal specimens, we were unable to detect CD18. We suggest that expression of the CD11b/CD18 heterodimer might facilitate transmission of HIV by enhancing binding of HIV–antibody complexes in seminal fluid to epithelial cells. Alternatively, since intercellular adhesion molecule-1 (ICAM-1) is a receptor for CD11b/CD18, this may promote adhesion between epithelial cells and HIV-infected mononuclear cells in seminal fluid.

Keywords CR3 (CD11b/CD18) HIV rectal mucosa adhesion molecule

INTRODUCTION

Transmission of HIV-1 commonly occurs through the rectal mucosa during rectal intercourse [1,2] or through the cervicovaginal mucosa during heterosexual intercourse [3,4]. The mechanism of transmission is not clear, especially the nature of cell surface receptors involved in HIV binding. Expression of the CD4 glycoprotein, which is the main receptor of HIV, has not been recorded by immunochemical staining of sections of human rectal [5] and with a few exceptions cervicovaginal epithelial cells [6]. Some HIV strains can infect cell lines of non-haematopoietic origin *in vitro*, and these do not usually express detectable levels of CD4 at the cell surface [7,8].

The involvement of cell surface receptors other than CD4 in HIV infection has been suggested by a number of studies. Antibody-dependent enhancement of infection of lymphoid cells involving Fcγ receptors has been demonstrated *in vitro* [9–11]. We have previously shown that both FcγR3 (CD16) and FcγR2 (CD32) are expressed by rectal epithelial cells [5] and by endocervical epithelial cells [6], and we raised the possibility that they may facilitate binding of HIV–antibody complexes. Cell adhesion molecules, including members of the integrin and immunoglobulin gene families, may be required for cell to cell transmission of HIV, since addition of MoAb directed against LFA-1 (CD11a) [12] or against CD18 (the common β chain of the leucocyte integrins) [12–15] to T cells or monocytes *in vitro* inhibited HIV-induced syncytium formation. Similarly, MoAb to intercellular adhesion molecule-1 (ICAM-1) (the receptor to LFA-1), inhibited syncytium formation [15], and although MoAb to CD2 had no effect when added to cell cultures, synergistic inhibition was observed when both MoAbs against LFA-1 and CD2 were used [13].

Transmission of HIV through the rectal or cervicovaginal mucosa may occur by cell-free or cell-associated virus, since both are present in seminal fluid [16,17]. The aim of this study was to determine whether cell adhesion molecules which may enhance HIV infection, could be detected in rectal or cervicovaginal epithelium.

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MATERIALS AND METHODS

Collection and preparation of tissue sections

Biopsies of rectal mucosa were obtained during surgery performed for diagnostic purposes, and 15 specimens that appeared clinically and histologically normal were selected. In addition, normal cervicovaginal 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. 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 gelatin-coated glass slides, and air-dried for 1 h. Sections of all samples were stained with haematoxylin and eosin for histological examination.

Monocolonal antibodies

MoAbs against CD18 and CD11a (LFA-1) were from Janssen Biochemica (Beerse, Belgium). MoAb against CD11b (OKM1) was purchased from Ortho-mune (High Wycombe, UK), and MoAbs against CR1 (CD35) and CR2 (CD21) were purchased from Becton Dickinson (Cowley, Oxford). The MoAb 6.5B5 against ICAM-1 was kindly provided by Dr D. Haskard (Department of Rheumatology, Hammersmith Hospital, London, UK).

Oligonucleotides

Biotinylated oligonucleotides corresponding to and complementary to nucleotides 2091–2135 of the cDNA sequence encoding CD18 [18] and corresponding to and complementary to nucleotides 2651–2695 of the cDNA sequence encoding CD11b [19] were purchased from Oswel DNA Services (University of Edinburgh, Edinburgh, UK).

Immunohistology

The indirect immunoperoxidase technique was applied, using the avidin-biotin-peroxidase complex method [20]. Tissue sections were fixed in acetone for 10 min, and were then incubated with optimal dilution of MoAbs 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, UK), or biotinylated goat anti-mouse IgM $F(ab)_2$ (Tago, Burlingame, CA) for 1 h. This was followed by incubation with 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 stained by incubating them in a solution of 3-3 diaminobenzidine (DAB) tetrahydrochloride (Sigma, Poole, UK) and hydrogen peroxide for 10 min [21]. 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. The immunoperoxidase preparations were examined independently by two of the authors (L.A.H. and T.L.), using a standard Zeiss microscope and Leitz Orthoplan microscope, respectively.

In situ *hybridization*

Cryostat sections (6 μm) of rectal biopsies were fixed in 4% paraformaldehyde for 25 min on slides coated with 3-aminopropyltriethoxysaline. Slides were then washed twice in PBS, dehydrated in graded alcohols, and used immediately or stored at −70°C. Before *in situ* hybridization, slides were treated by washing in PBS which contained 0·5 mM $MgCl_2$ followed by PBS which contained 0·5 mM $MgCl_2$ and 0·1 M glycine. For N-acetylation, slides were equilibrated in 0·1 M triethanolamine–HCl pH 8·0 for 15 min before treatment with 0·25% (v/v) acetic anhydride in the same buffer for 5 min. Subsequently slides were washed twice with 2 × SSPE (SSPE is 0·15 M NaCl, 1 mM EDTA, 0·01 M NaH_2PO_4, pH 7·4) for 5 min and twice with 50% formamide (Sigma) in 2 × SSPE for 10 min at 45°C. Slides were dried and treated with hybridization solution (50% formamide, 1 × Denhardt's solution, 100 mM DTT, 0·05% SDS, 0·5 mg/ml sheared salmon sperm DNA, 0·5 mg/ml wheat germ t-RNA in 2 × SSPE) for 3 h at 45°C and then dehydrated in alcohol. The tissue sections were then incubated with biotinylated oligonucleotide probes (5 nM) in hybridization solution for 16 h at 45°C to allow hybridization. The slides were then washed briefly in 4 × SSPE followed by 0·1 M DTT, 1 mM EDTA in 4 × SSC (SSC is 0·15 M NaCl in 0.015 M Na citrate pH 7·0) for 2 h at room temperature and in 50% (v/v) formamide, 2 × SSPE, 10 mM DTT in 20 mM Tris HCl pH 7·4 for 8 min at 60°C. After two further washes (15 min at room temperature) in 0·1 × SSC, slides were dried and sections were overlaid with streptavidin–horseradish peroxidase conjugate (Dakopatts) in 0·5 M Tris HCl pH 7·6. Incubation was for 90 min at room temperature in a moist chamber. Slides were washed three times in TBS, and hybridizing oligonucleotides were visualized by incubation in DAB tetrahydrochloride (Sigma) and hydrogen peroxide for 10 min. After washing three times in TBS, sections were counterstained with haematoxylin, dehydrated in graded alcohol, cleared in xylene and mounted in DPX. Control sections were incubated with sense oligonucleotides or were treated with RNAase before hybridization.

RESULTS

Expression of CD18 in rectal and cervicovaginal epithelium

Biopsies from clinically and histologically normal rectal tissues showed CD18, the common β chain of the leucocyte integrins, in the surface epithelial (four out of 15 rectal biopsies) and crypt epithelial cells (eight out of 15 biopsies) (Fig. 1c). Both basolateral and apical surfaces of the epithelial cells were stained. Mononuclear cells in the lamina propria reacted with CD18 antibodies in all specimens. Sections of cervicovaginal tissue

Table 1. Immunohistological findings of cell adhesion molecules in human rectal ($n = 15$) and cervicovaginal ($n = 14$) tissues

	Rectal tissue		Cervicovaginal tissue	
Receptor	Epithelium	Lamina propria	Epithelium	Stroma
CD18 (β_2)	8*	15	0†	14
CD11b (Mac-1α)	10	6	3†	5
CD11a (LFA-1α)	1†	15	0†	14
CD54 (ICAM-1)	0	15	0	14

* Seven of eight of these biopsies also stained with anti-CD11b MoAb.

† The staining was confined to endocervical epithelial cells; mononuclear cells infiltrating the epithelium were positive for CD18 and CD11b, CD11a.

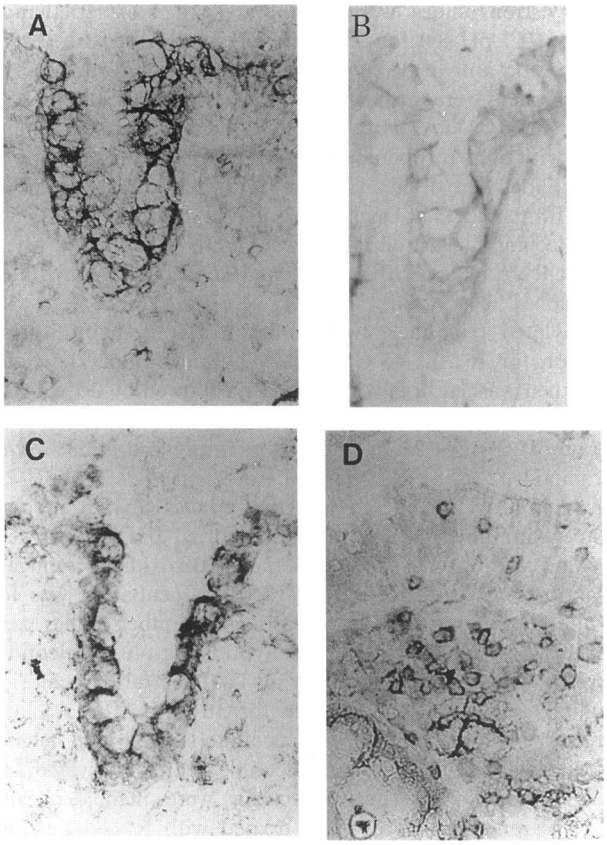

Fig. 1. Immunoperoxidase staining of rectal epithelium. (A) CD11b in surface and crypt epithelia. (B) No staining by omitting the MoAb. (C) CD18 in crypt epithelium. (D) CD11a staining of mononuclear cells infiltrating the epithelium and lamina propria (×250).

were also examined, but no staining of the epithelium was observed (Table 1), although mononuclear cells in the cervical stroma and vaginal lamina propria adjacent to the epithelium were stained. Some mononuclear cells infiltrating the cervical and vaginal epithelium were also positive for CD18.

Expression of CD11b and CD54 in rectal and cervicovaginal epithelium

The integrin chain which is associated with CD18 was then investigated. In only one out of 15 rectal specimens was LFA-1 (CD11a) detected on the surface and crypt epithelia, although mononuclear cells of lamina propria, some of which were infiltrating the epithelium, were strongly positive for LFA-1 (Table 1, Fig. 1D). Similarly, LFA-1 was not detected in cervicovaginal epithelium, but was present in mononuclear cells of the cervical stroma adjacent to the epithelium, as well as in the cells infiltrating the ectocervical squamous epithelium and the endocervical columnar epithelium. The Mac-1 (CD11b) antigen was, however, detected in 10 out of 15 rectal biopsies (using MoAb OKM1) and staining was predominantly of the crypt epithelium (Fig. 1A). As with CD18, both apical and basolateral surfaces of the epithelial cells were stained. Mononuclear cells in the lamina propria were also stained. It should be noted that seven out of 15 rectal specimens which were positive for CD18 were also positive for CD11b. Of the cervicovaginal biopsies, only three out of 14 were positive for CD11b in the endocervical epithelium, and none expressed CD18. The intensity of staining was, however, lower than that in the rectal specimens.

CR1 and CR2 have not been detected in any epithelial cells using MoAb to CD35 and CD21, respectively. Expression of ICAM-1 was also investigated in rectal and cervicovaginal biopsies, and although cells of the lamina propria, especially endothelial cells, were stained, ICAM-1 was not detected in the epithelium. As routine controls for specificity in all experiments, MoAbs were omitted before addition of the second layer biotinylated antiserum (Fig. 1B).

Detection of CD18 and CD11b gene transcripts in rectal epithelium

To provide further evidence that CD18 and CD11b may be expressed in rectal epithelial cells, tissue sections from five specimens which were positive when probed with MoAbs were analysed for the presence of gene transcripts by *in situ* hybridization using biotinylated oligonucleotide probes. Staining of surface and crypt epithelial cells was evident when sections were probed with anti-sense oligonucleotides derived from the cDNA sequences of CD18 and CD11b [18,19] (Fig. 2A,C). In addition, some mononuclear cells in the lamina propria were stained. No staining was apparent in sections treated with RNAase before hybridization (Fig. 2B,D) which confirmed the specificity of hybridization. Similarly, no staining was observed when sections were probed with sense oligonucleotides (result not shown).

DISCUSSION

The integrins form a family of cell surface proteins involved in cell–cell and cell–extracellular matrix interactions [22]. They are $\alpha\beta$ heterodimers and are further grouped into subfamilies, members of each subfamily sharing a common β subunit. The leucocyte integrin subfamily (the β_2 integrins) comprises Mac-1, LFA-1 and p150,95, and exhibits a more restricted tissue distribution than do the other subfamilies [23]. Expression of Mac-1 (CD11b/CD18) has previously been reported to be restricted to myeloid and natural killer cells [22]. The results of this study from both immunohistology and *in situ* hybridization suggest that CD11b/CD18 is also expressed in the colorectal epithelial cells. Transcripts of both genes were detected in five selected specimens which also stained positively with anti-CD18 and anti-CD11b MoAbs. Further evidence for this proposal was provided by the observation that three out of five colorectal cell lines stained with the CD11b MoAb (result not shown). The immunohistological studies of cervicovaginal tissue suggest that CD11b is expressed in some specimens of endocervical epithelium (3/14), although the level of expression appears to be lower than that found in rectal tissue. Although lower levels of expression may also explain the lack of reaction with MoAb against CD18 observed in the cervicovaginal tissue, we cannot be confident of the true nature of CR3 in this epithelium.

The CD11b/CD18 complex functions as both a complement receptor (CR3) for the iC3b component and as a cell adhesion molecule [24]. Either or both of these functions might contribute to rectal transmission of HIV. Several components of the complement system, including C3, are present in seminal fluid [25–27], as are IgG and IgA [28]. In seminal fluid from healthy

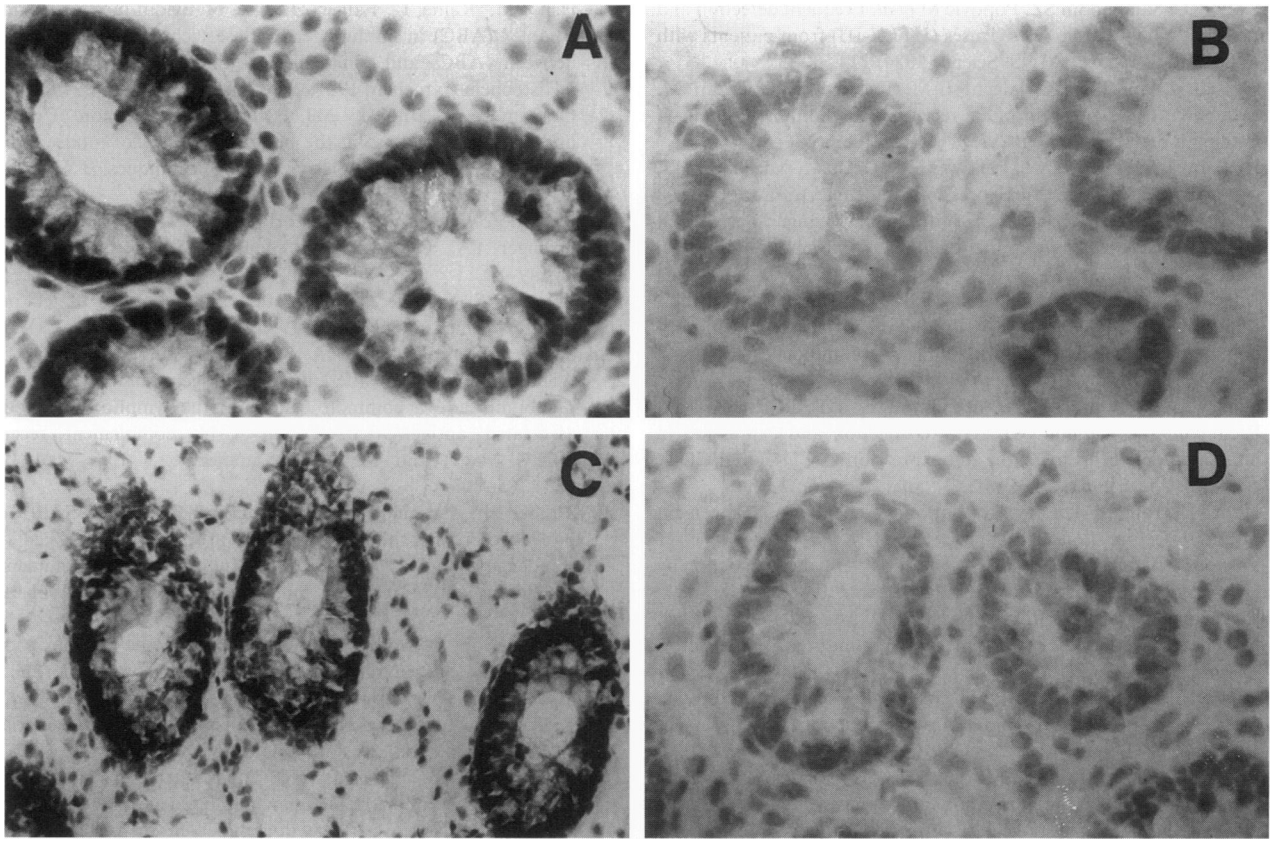

Fig. 2. Detection of CD11b and CD18 gene transcripts in rectal epithelium. The presence of mRNA was determined by *in situ* hybridization using biotinylated (antisense) oligonucleotide probes for CD11b (A,B) and CD18 (C,D). Control sections were treated with RNAase before hybridization (B,D) (×660).

individuals, concentrations of C3 are low (<1 mg/dl), but elevated levels of C3 are found in seminal fluid from individuals with prostatic infection which is common in promiscuous homosexual and heterosexual men [28]. Isolated HIV particles can activate complement by the classical pathway [29] and to a lesser extent by the alternative pathway [30]. Complement activation in seminal fluid may therefore lead to deposition of C3b on the surface of virus particles which are free or bound by antibody. CD46 (membrane cofactor protein) is present in soluble form in seminal fluid [27] and is associated with the membrane of HIV [31], and thus may act as a cofactor for factor I-mediated cleavage of C3b to iC3b, which is the ligand for CR3. Moreover, CR3 has been shown to mediate antibody-independent infection of $CD4^+$ and $CD4^-$ human monocyte cell lines cultured with complement-opsonized HIV [32]. We suggest therefore that iC3b deposition on free HIV or HIV–antibody complexes in seminal fluid may lead to internalization of virus into rectal epithelial cells, following attachment to CR3.

Since ICAM-1 is a ligand for CD11b/CD18 (Mac-1) [33], enhancement of infection with cell-associated HIV might also be mediated by interaction between Mac-1 on epithelial cells and ICAM-1 on HIV-infected CD4 lymphocytes or monocytes in seminal fluid.

Possible mechanisms for CD4-independent HIV transmission proposed in this study and others [5–11,30,32] include antibody-dependent or -independent enhancement of infection with cell-free virus which may be complement-mediated. Integrin-mediated attachment may facilitate cell-associated virus infection. It should, however, be emphasized that there is as yet no direct proof for these mechanisms in HIV infection *in vivo*.

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