European Journal of Immunology

Blocking of integrins inhibits HIV-1 infection of human cervical mucosa immune cells with free and complement-opsonized virions

Veronica Tjomsland[*1], Rada Ellegård*[*1], Preben Kjölhede[2], Ninni Borendal Wodlin[2], Jorma Hinkula[1], Jeffrey D. Lifson[3] and Marie Larsson[1]*

[1] Division of Molecular Virology, Department of Clinical and Experimental Medicine, Linköping University, Linköping, Sweden
[2] Division of Gynecology, Department of Clinical and Experimental Medicine, Linköping University, Linköping, Sweden
[3] SAIC/Fredrick, National Cancer Institute at Fredrick, Frederick, Maryland, USA

The initial interaction between HIV-1 and the host occurs at the mucosa during sexual intercourse. In cervical mucosa, HIV-1 exists both as free and opsonized virions and this might influence initial infection. We used cervical explants to study HIV-1 transmission, the effects of opsonization on infectivity, and how infection can be prevented. Complement opsonization enhanced HIV-1 infection of dendritic cells (DCs) compared with that by free HIV-1, but this increased infection was not observed with $CD4^+$ T cells. Blockage of the α4-, β7-, and β1-integrins significantly inhibited HIV-1 infection of both DCs and $CD4^+$ T cells. We found a greater impairment of HIV-1 infection in DCs for complement-opsonized virions compared with that of free virions when αM/β2- and α4-integrins were blocked. Blocking the C-type lectin receptor macrophage mannose receptor (MMR) inhibited infection of emigrating DCs but had no effect on $CD4^+$ T-cell infection. We show that blocking of integrins decreases the HIV-1 infection of both mucosal DCs and $CD4^+$ T cells emigrating from the cervical tissues. These findings may provide the basis of novel microbicidal strategies that may help limit or prevent initial infection of the cervical mucosa, thereby reducing or averting systemic HIV-1 infection.

Keywords: $CD4^+$ T cells · Complement system · DCs · HIV · Integrins

Additional supporting information may be found in the online version of this article at the publisher's web-site

Introduction

The most common route of HIV-1 transmission is by hetero-sexual intercourse, with women accounting for more than half of the newly HIV-1-infected individuals [1]. During sexual transmission, the first interactions between HIV-1 and the host occur at mucosal sites, such as genital and rectal mucosa. Within hours of initial exposure, the virions can reach the submucosa and local events that contribute to the establishment of a systemic infection are set in motion [2], leading to high levels of infection and destruction of $CD4^+$ T effector memory cells, particularly in the gastrointestinal tract [3]. While immune cells can be found at

Correspondence: Prof. Marie Larsson
e-mail: marie.larsson@liu.se

*These authors contributed equally to this work.

© 2013 The Authors. European Journal of Immunology published by WILEY-VCH Verlag GmbH & Co. KGaA Weinheim.
This is an open access article under the terms of the Creative Commons Attribution-NonCommercial License, which permits use, distribution and reproduction in any medium, provided the original work is properly cited and is not used for commercial purposes.

sites of inflammation throughout the female genital tract, in the absence of such inflammation, immune cells are found most frequently in the cervical transformation zone and surrounding tissues and are likely sites for HIV infection [4, 5].

Studies in macaques inoculated with SIV, to model vaginal transmission of HIV, have shown that SIV virions cross the single layer mucosal barrier of the endocervix, however, virus has also been shown to enter through the stratified epithelium [6], especially at sites of preexisting inflammation. Sexually transmitted diseases and genital infections increase the risk of acquiring HIV-1 infection due to disturbed epithelial layer and increased availability of target cells in the mucosa; moreover, the production of proinflammatory cytokines, such as TNF-α, is associated with an enhanced HIV-1 replication [7].

Another potential mechanism available to the virus to penetrate the mucosa is by exploiting dendritic cells (DCs) and Langerhans cells (LCs) localized in the stratified epithelium, where these cells can pick up HIV-1 and transfer the virus to the submucosa [8]. When HIV-1 has reached the submucosa, the primary target cells for the virions are $CD4^+$ T cells, macrophages, and DCs. In addition to binding to CD4 and the coreceptor CCR5/CXCR4 [9], HIV-1 can be captured by DCs via an array of cell surface receptors, such as heparin sulfate proteoglycans, C-type lectins, and integrins [9–11]. As soon as 24 h postexposure, DCs with captured virions can reach draining LNs where they can transfer infectious HIV-1 to surrounding cells [2, 12]. In macaques challenged vaginally with SIV, small founder populations of productively infected cells are demonstrable in the cervical mucosa 3 to 4 days postexposure. The founder populations normally consist of resting $CD4^+$ T cells with a receptor pattern and functionality that support a productive infection [13, 14], i.e. $CD4^+$ T cells with high expression of CCR5 and α4β7-integrins [15]. After local amplification, the infection spreads to draining and then distant lymphoid tissues, resulting in a disseminated infection [6, 13].

The mucosal epithelium is not a simple passive barrier; it also mediates host defenses by secreting mucus, defensins, and complement proteins [16]. HIV-1 can activate the different complement cascade pathways, which all converge at the cleavage of C3, but HIV-1 can escape complement-mediated lysis as the virions incorporate host proteins that will cleave activated C3 into inactivated iC3b [17]. The iC3b fragments are covalently attached to the viral protein gp120 and can interact with complement receptor 3 (CR3: CD11b/CD18, αMβ2 integrin). After seroconversion, virions are covered with both specific antibodies and complement [17]. In addition, seroconversion enhances complement activation and increases the amount of C3 cleavage products on the surface of HIV-1 [18].

Complement-opsonized virions already exist at the mucosal surface during the initial phase of infection [18]. We have shown earlier that complement-opsonized HIV-1 is internalized more efficiently by DCs than free HIV-1 [9]. Complement opsonization of HIV-1 has also been shown to enhance in vitro infectivity for cultured monocytes [19] and immature DCs [20–22]. Given the potential impact of opsonization of HIV-1 on both the acute and chronic phases of infection, it is important to understand the effects of opsonization on the ability of HIV-1 to interact with cells and to infect them. The aim of this study was to investigate the initial HIV-1 infection of cervical mucosa, using an ex vivo cervical tissue explant model, comparing free and opsonized virions. The degree of infection of mucosal DCs and $CD4^+$ T cells, and the receptors involved in the initial infection were analyzed.

Complement opsonization of virions resulted in a higher level of infection in DCs emigrating from explant tissues in culture compared with that of free HIV-1, whereas the effect on infection of emigrating $CD4^+$ T cells was the opposite with decreased infection for opsonized HIV-1. Blocking of CD4, and integrins β1, β2, α4, and β7 all resulted in significantly decreased infection of DCs and $CD4^+$ T cells. We found a higher impairment in HIV-1 infection in emigrating DCs for complement-opsonized virions when interactions with αM/β2- and α4-integrins were blocked than for free virions. Blockade of integrins decreased both the infection of DCs and $CD4^+$ T cells emigrating from the cervical explant tissues, suggesting novel approaches for microbicidal strategies to limit or prevent vaginal transmission of HIV.

Results

Complement opsonization of HIV-1 enhances infection of DCs emigrating from cervical mucosa tissue

We studied the initial phase of HIV-1 infection in cervical mucosa. Cervical tissues were challenged with $HIV\text{-}1_{BaL}$ in different forms: free HIV-1 (F-HIV); complement opsonized HIV-1 (C-HIV), immune complexed HIV-1 with a mixture of unspecific, and HIV-1 specific neutralizing IgG (IgG-HIV); or HIV-1 opsonized with both complement and a mixture of unspecific and HIV-1 specific neutralizing IgG (C-IgG-HIV) and infection was measured on day 3–6. The level of HIV-1 infection of emigrating DCs was highest for C-HIV and C-IgG-HIV (Fig. 1A), whereas F-HIV gave the highest level of infection in $CD4^+$ T cells (Fig. 1B). Compared with F-HIV, normalized as 100% value for infection, both C-HIV (151%: $p < 0.005$) and C-IgG-HIV (141%) showed enhanced infection of DCs emigrating from cervical explants (Fig. 1C), while infection using IgG-HIV was significantly decreased (60%; $p < 0.005$) (Fig. 1C). However, HIV-1 infection of mucosal $CD4^+$ T cells emigrating from explant cultures was significantly decreased when exposed to opsonized forms of virions (C-HIV (80%, $p = 0.01$), IgG-HIV (70%, $p = 0.025$), or C-IgG-HIV (64%, $p = 0.026$) compared with F-HIV (normalized to 100%) (Fig. 1D). The infection profile was the same for endocervix and ectocervix (Supporting Information Fig. 1A and B).

The infection profiles were similar, independently of whether cultures were harvested at day 3, 5, or 6 (Fig. 1E and F) with the greatest difference in the level of infection in DCs using F-HIV versus C-HIV seen at day 5 (Fig. 1E). Impairment of T-cell infection by virion opsonization was more pronounced at day 3 than at day 6 (Fig. 1F). To assess the infection at a later time point than day 3–6, it was necessary to add exogenous GM-CSF and IL-2 to the culture to maintain cell viability. We

© 2013 The Authors. European Journal of Immunology published by WILEY-VCH Verlag GmbH & Co. KGaA Weinheim.

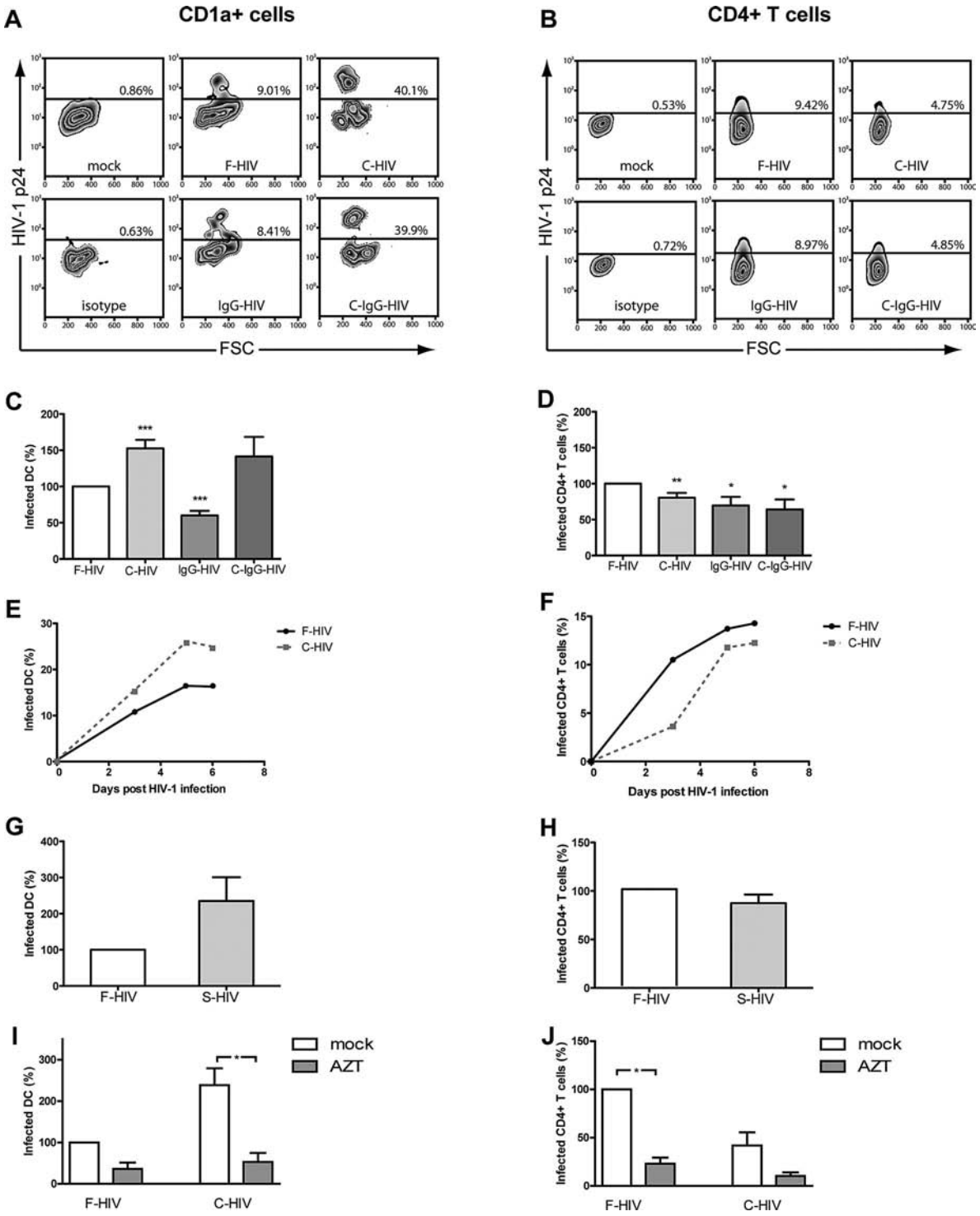

Figure 1. Complement opsonization of HIV-1 enhances infection of DCs but decreases infection of $CD4^+$ T cells. The cervical tissue biopsies were infected with different forms of HIV-1_{BaL}, either free (F-HIV), complement opsonized (C-HIV), antibody opsonized (IgG-HIV), virions opsonized by a cocktail of complement and antibodies (C-Ig-HIV) or mock-treated, by spinning the cultures for 2 h at 37°C. The tissues were washed and transferred to six-well plates and cultured for 3–6 days. (A, B) The emigrating cells were harvested and stained with (A) anti-CD1a and anti-HIV-1 mAbs for DCs, and (B) anti-CD3, anti-CD4, and anti-HIV-1 mAbs for $CD4^+$ T cells and the level of infection was assessed by flow cytometry. (C, D) The level of HIV-1 infection in the different (C) DC ($N = 10–32$) or (D) T-cell ($N = 11–32$) experiments was normalized with F-HIV set as 100%. (E, F) The kinetics of HIV-1 infection of emigrating (E) DCs and (F) $CD4^+$ T cells were measured day 3–6. (G, H) The level of HIV-1 infection of emigrating (G) DCs and (H) T cells ($N = 4–5$) exposed to F-HIV or HIV-1 opsonized with seminal fluid was also assessed. (I, J) The level of HIV-1 infection of emigrating (I) DCs and (J) T cells from cervical tissue biopsies ($N = 3$) pre-exposed to mock or AZT followed by infection with F-HIV or C-HIV was measured on day 4. Mock or AZT was present throughout the whole 6-day culture. Statistical significance was tested using a two-sided paired *t*-test. $^*p < 0.05$, $^{**}p < 0.001$, $^{***}p < 0.0001$. Data are shown as mean + SEM of the indicated number of samples, each assessed in its own experiment.

© 2013 The Authors. European Journal of Immunology published by WILEY-VCH Verlag GmbH & Co. KGaA Weinheim.

found the same profile but enhanced infection at day 8 in both DCs and T cells compared with day 3–6 (Supporting Information Fig. 1C).

To further enhance the potential in vivo relevance, we performed similar studies using fresh seminal fluid as the opsonizing agent. The seminal fluid gave similar results as the fresh blood serum with an increased infection of DCs (211%) and a decreased infection of T cells (74%) for opsonized versus nonopsonized virions (Fig. 1G and H). Infection of cervical tissues was also assessed with two additional HIV-1 strains, the CXCR4 tropic HIV-MN and CCR5 tropic HIV-ADA, but these viruses gave very low or no infection (Supporting Information Fig. 1D) and this is in accordance with findings by Greenhead et al. [23]. To distinguish between productive infection of the DCs and $CD4^+$ T cells and p24 immunostaining of internalized virions without productive infection, experiments were performed where the reverse transcriptase inhibitor azidothymidine (AZT) was present throughout the whole course of culture. Tissues exposed to AZT had decreased levels p24 gag positive cells compared to untreated HIV-1-infected cells (Fig. 1I and J) confirming that most of the p24 signal was attributable to productive infection. To further characterize HIV infection of cervical mucosa, we assessed the levels of HIV-1 p24 in the supernatants at day 4 and we found that C-HIV gave a higher infection compared with the level obtained with F-HIV (Supporting Information Fig. 1E).

Characterization of C-type lectin and integrin expression on cervical mucosa DCs and T cells

To better understand the establishment of HIV-1 infection in the cervical mucosa, we characterized the expression of an array of receptors, and the location of DCs and T cells. The expression and both cellular and anatomic localization of the C-type lectin receptors MMR (CD206), DC-SIGN (CD209), and Langerin (CD207), and integrins β1, β2, β7, α4, and αM were assessed by flow cytometry (for emigrating cell populations) and fluorescence microscopy (for cervical tissues). Expression of Langerin was detected almost exclusively on LCs located in the epithelium (Fig. 2A). The vast majority of $CD3^+$ T cells were located within the lamina propria (LP), but a few T cells could be found in the epithelium (Fig. 2B). CD1a expression was detected both on the LCs in the epithelial layer and on submucosal DCs in the LP (Fig. 2C). MMR expression was only detected in cells in the LP, presumably on submucosal DCs and macrophages (Fig. 2D). Cells expressing αM/β2-intergrins were located both in the epithelium and LP, and likely consist of LCs in the epithelial layer and submucosal DCs and macrophages in LP (Fig. 2E). β1-integrin expression was found on cells in LP and on the basement membrane (Fig. 2F), while cells expressing the β7-integrin were detected in the LP (Fig.2G). The specificity of all positive staining was verified by use of isotype control antibodies (Fig. 2H and I). Emigrating DCs and T cells were assessed for expression of C-type lectin receptors MMR, DC-SIGN, and Langerin, and integrins β1, β2, β7, α4, and αM. Expression of C-type lectin receptors MMR, DC-SIGN, or Langerin was detected on DCs (Fig. 2J) but not on T cells (our unpublished observation). β1, β2, β7, α4, and αM integrins were expressed by DCs (Fig. 2J), whereas $CD4^+$ T cells only expressed β1, β7, and α4 (our unpublished observation).

Blockage of CD4 binding abolishes infection of DCs and $CD4^+$ T cells by free and opsonized HIV-1

We investigated the infection in DCs and $CD4^+$ T cells after blocking CD4 with the mAb b12, a neutralizing antibody that specifically targets the CD4 binding site on gp120, and CCR5 with the inhibitor TAK779. We mostly used the b12 experiment to establish the level of inhibition reached in this model by a known blocker of HIV-1 infection [24]. b12 significantly decreased the infection of migrating DCs to 45% of unblocked control for F-HIV ($p = 0.019$) and to 62% of control for C-HIV ($p = 0.028$) (Fig. 3A). When b12 was used, infection of $CD4^+$ T cells emigrating from cervical explants was decreased by 85% ($p < 0.0001$) for F-HIV and by 74% ($p < 0.0001$) for C-HIV (Fig. 3B). The CCR5 inhibitor TAK799 decreased the HIV-1 infection of emigrating DCs by 41% ($p = 0.037$) for F-HIV and 66% for C-HIV ($p = 0.06$) and of $CD4^+$ T cells by 48% ($p = 0.02$) for F-HIV and 68% ($p = 0.01$) for C-HIV (Fig. 3C and D).

Preventing HIV-1 binding to MMR inhibits infection in emigrating cervical DCs

To study the impact of C-type lectins on infection in DCs and $CD4^+$ T cells, we exposed cervical mucosa to the mannose polymer mannan, a competing ligand for C-type lectins, before challenging the tissues with HIV-1. Infection was decreased for both F-HIV (46%, $p = 0.005$) and C-HIV (40%, $p = 0.023$) in emigrating DCs after blocking C-type lectins located in the cervical mucosa (Fig. 4A). In contrast, no effect on the infection of emigrating $CD4^+$ T cells was observed (Fig. 4B). DCs emigrating from cervical tissues have been shown to express MMR [24]. Blocking this C-type lectin using anti-MMR mAb gave similar results as when C-type lectins were blocked using mannan with a 51% ($p = 0.006$) decrease in F-HIV infection in DCs (Fig. 4C). When the cervical tissue was challenged with C-HIV, the corresponding decrease in DC infection was 43% ($p = 0.029$) (Fig. 4C). As expected, infection of $CD4^+$ T cells was not affected by blocking MMR in the cervical mucosa (Fig. 4D). Blocking of MMR had a slightly higher effect on DC infection with F-HIV than with C-HIV.

Blockade of integrins decreases HIV-1 infection in emigrating DCs and $CD4^+$ T cells

Integrins are used by different viruses to attach or infect host cells [25]. Many integrins are expressed by DCs and T cells and we investigated the role of the αM (CD11b) and β2 (CD18) integrins, i.e. CR3, expressed on both DCs and LCs, in the establishment

© 2013 The Authors. European Journal of Immunology published by WILEY-VCH Verlag GmbH & Co. KGaA Weinheim.

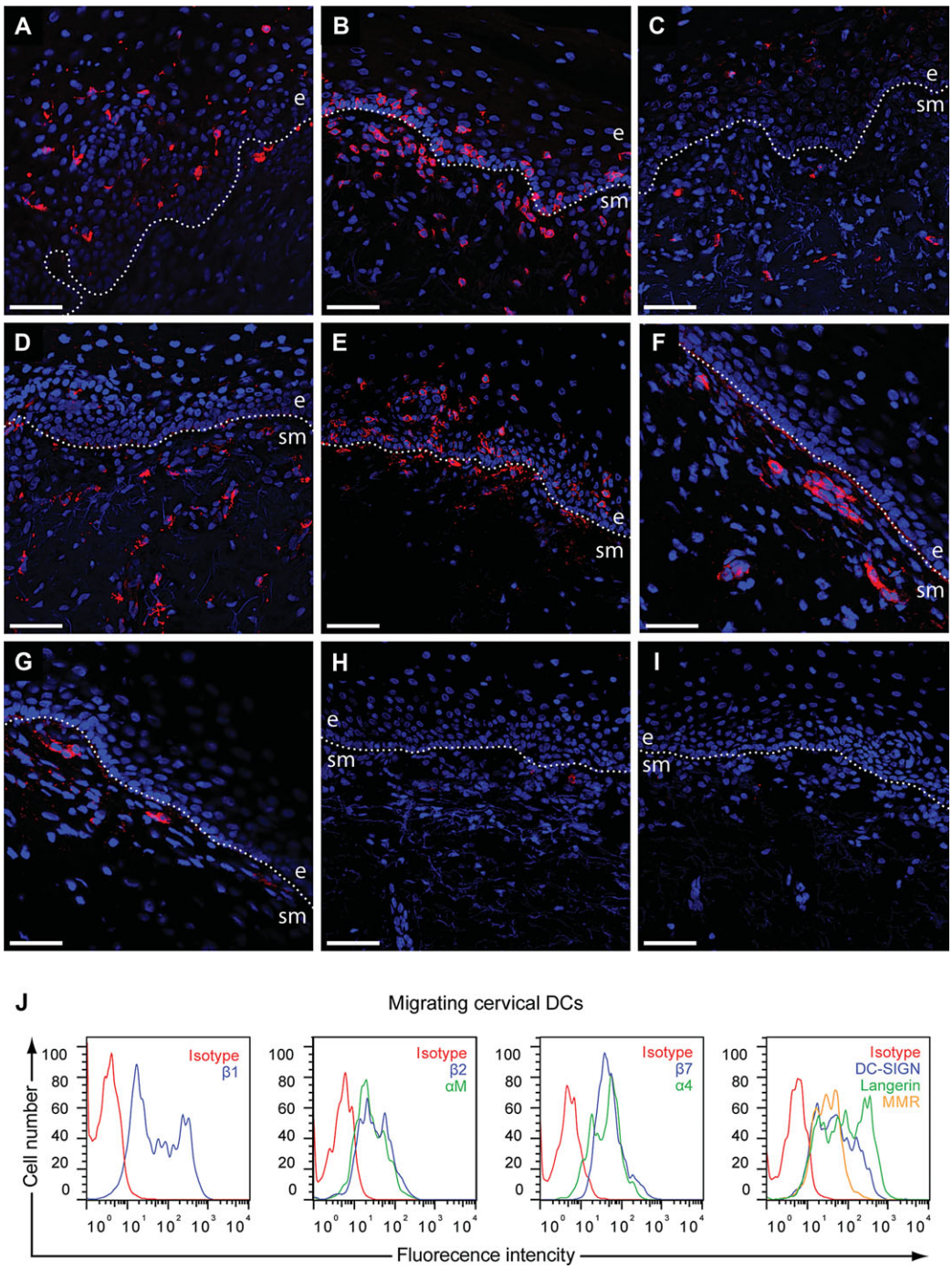

Figure 2. Expression of C-type lectins and integrins in cervical mucosa tissue and on emigrating immune cells. (A–I) Cryopreserved cervical mucosa tissue sections were stained with (A) anti-Langerin, (B) anti-CD3, (C) anti-CD1a, (D) anti-MMR, (E) anti-CD11b/CD18, (F) anti-β1, (G) anti-β7, or (H, I) isotype control. The primary antibody was visualized by an sAb conjugated to Rhodamin Red-X. The sections were mounted using mounting medium for fluorescence containing DAPI. Receptor expression is shown in red in the cervical mucosa, scale bar, 50 μm. (J) DCs emigrating from cervical mucosa tissue were labeled with anti-Langerin, anti-CD3 anti-DC-SIGN anti-MMR, anti-CD11b, anti-β1, anti-β7, anti-α4, or isotype control followed by goat-anti-mouse Alexa Fluor 488 and analyzed by flow cytometry. e = epithelium, sm = submucosa. Data shown are representative of three to five stainings performed on different tissue samples.

of HIV-1 infection. Cervical mucosa was incubated with anti-αM integrin and anti-β2 integrin mAbs to block CR3 before adding F-HIV or C-HIV. Upon blockade of CR3, the infection of emigrating DCs was decreased by 54% for F-HIV and 82% for C-HIV (p = 0.023) (Fig. 5A). The infection in emigrating $CD4^+$ cells was also decreased but to a lesser extent. Blocking CR3 inhibited infection in the $CD4^+$ T-cell population for F-HIV with 38% (p = 0.021) and C-HIV with 54% (p = 0.001) (Fig. 5B).

© 2013 The Authors. European Journal of Immunology published by WILEY-VCH Verlag GmbH & Co. KGaA Weinheim.

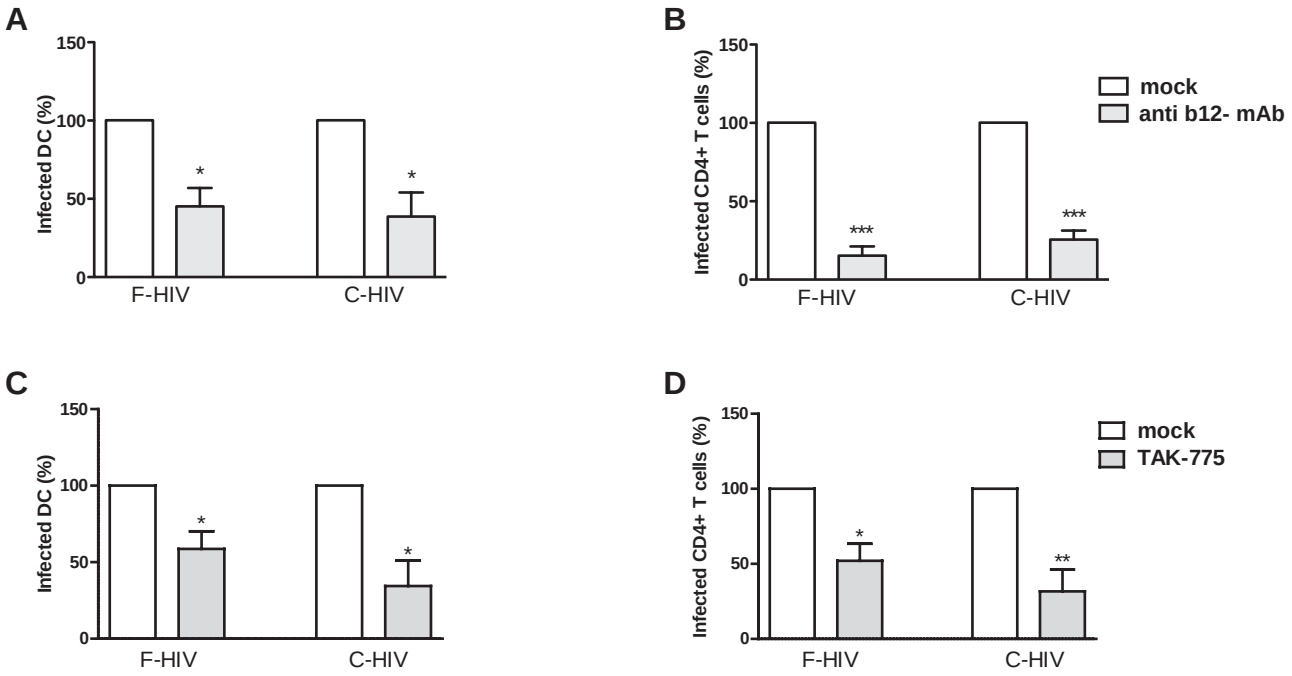

Figure 3. Blockade of CD4 and CCR5 decreases the infection of emigrating cervical mucosa DCs and T cells. Cervical tissue biopsies were infected with HIV-1_{BaL}, either as free virions (F-HIV) or complement opsonized virions (C-HIV) that had been mock treated or incubated with b12 mAb, for 2 h at 37°C.The cervical tissue biopsies were pretreated for 30 min at 37°C with TAK779 followed by infection with different forms of HIV-1_{BaL}, either free (F-HIV) or complement opsonized (C-HIV), for 2 h at 37°C. The tissues were washed and transferred to six-well plates and cultured for 3–6 days in the absence or continuous presence of b12 mAb or TAK779. The emigrating cells were harvested and stained with anti-CD1a, and anti-HIV-1 mAbs for DCs and anti-CD3, anti-CD4, and anti-HIV-1 mAbs for $CD4^+$ T cells and level of infection was assessed by flow cytometry for HIV-1 p24 expression. (A, B) The level of HIV-1 infection after mock treatment or treatment with b12 in the different (A) DCs ($N = 4$–6) or (B) T cells ($N = 4$–7) was normalized with mock-treated F-HIV set as 100%. (C, D) The level of HIV-1 infection after mock or TAK779 treatment in the different (C) DC or (D) T-cell experiments was normalized with F-HIV set as 100% ($N = 3$–4). Statistical significance was tested using a two-sided paired *t*-test. $^*p < 0.05$, $^{**}p < 0.001$, $^{***}p < 0.0001$. Data are shown as mean + SEM of the indicated number of samples, each assessed in its own experiment.

α4- (CD49d), β1- (CD29), and β7-integrins are expressed by DCs (Fig. 2) and T cells in the cervical mucosa in different heterodimeric complexes, and α4β7-, αEβ7-, and α4β1-integrins are expressed on T cells [26], while αEβ7- and α4β1-integrins are expressed on DCs. We investigated whether blocking β1-integrins would affect the infection of emigrating immune cells from cervical tissue after exposure to free or complement opsonized HIV-1. Pretreatment of the cervical mucosa with anti-β1 integrin mAb inhibited infection in both emigrating DCs and $CD4^+$ T cells. Infection of DCs was inhibited by 66% ($p = 0.008$) for F-HIV and by 57% for C-HIV ($p = 0.013$) (Fig. 5C). In $CD4^+$ T cells, the blocking of β1-integrins gave a 49% decrease in infection for F-HIV ($p = 0.01$) and a 46% decrease for C-HIV ($p = 0.01$) (Fig. 5D). HIV-1 gp120 binds to α4β7-integrins expressed on T cells and facilitates the formation of virological synapses and spread of the virus via LFA-1 [26], whereas the effects of virion interaction with α4 and β7 on DCs remain unknown. Targeting the integrins α4 or β7 in the cervical mucosa with specific blocking antibodies [27–29] resulted in a decreased infection in both emigrating DCs and $CD4^+$ T cells (Fig. 5E–H). Blocking the α4 integrin decreased the infection in DCs by F-HIV by 62% ($p = 0.006$) and by C-HIV by 80% ($p = 0.006$) (Fig. 5E). In the $CD4^+$ T-cell population, infection was decreased by 38% ($p = 0.022$) for F-HIV and 55% ($p = 0.0025$) for C-HIV (Fig. 5F). The blocking of β7-integrins in the cervical tissue also decreased infection in the emigrating DCs by 49% ($p = 0.012$) for F-HIV and 66% ($p = 0.001$) for C-HIV (Fig. 5G). The infection of CD4+ T cells was inhibited by 44% ($p = 0.004$) for F-HIV and by 58% ($p = 0.004$) for C-HIV (Fig. 5H). We found the same level of inhibition of infection when a lower concentration (5 μg/mL compared with 20 μg/mL) of the anti-β7 integrin or anti-α4 integrin antibodies was used (Supporting Information Fig. 2).

Of note, areas positive for HIV-1 p24 were detected in the submucosa tissues in all cervical tissues infected with F-HIV or C-HIV, whereas these areas were rarely or not found when the use of α4 and β7-integrins had been inhibited (our unpublished observation).

Discussion

It is important to gain a better understanding regarding the initial events in HIV-1 infection in the female genital tract in order to develop new strategies that prevent the initial infection or target the first week of local infection, with the ability to block the development of systemic infection. When studying the first steps of HIV-1 infection, there are many layers of interactions occurring within the mucosal microenvironment to take into account,

© 2013 The Authors. European Journal of Immunology published by WILEY-VCH Verlag GmbH & Co. KGaA Weinheim.

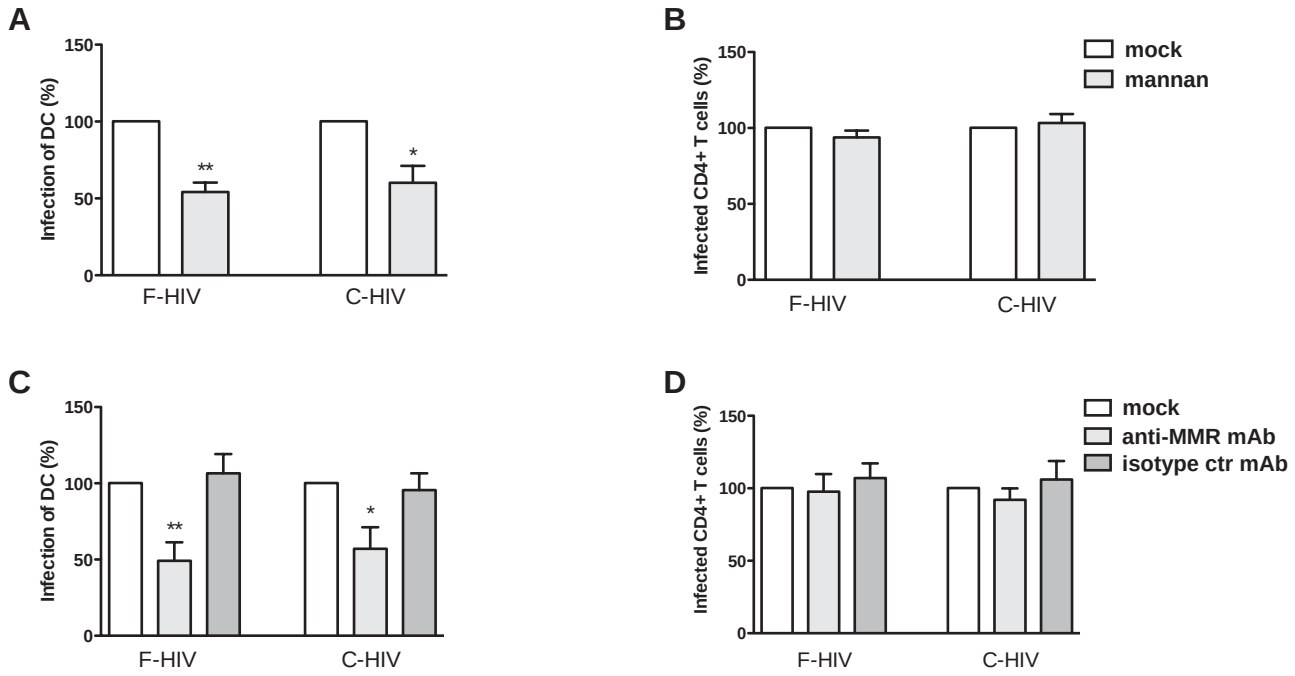

Figure 4. Blockade of the C-type lectin MMR decreases the infection of DCs emigrating out from cervical tissues. The cervical tissue biopsies were pretreated for 30 min at min 37°C with mock, mannan, anti-MMR mAb, or isotype control mAb followed by infection with different forms of HIV-1_{BaL}, either free (F-HIV) or complement opsonized (C-HIV), by spinning the cultures for 2 h at 37°C. The tissues were washed and transferred to six-well plates and cultured for 3–6 days with mock, mannan, anti-MMR mAb, or isotype control mAb. The emigrating cells were harvested and stained with anti-CD1a, and anti-HIV-1 mAbs for DCs and anti-CD3, anti-CD4, and anti-HIV-1 mAbs for $CD4^+$ T cells and level of infection was assessed by flow cytometry. (A, B) The level of HIV-1 infection in (A) DCs ($N = 4–5$) or (B) T cells ($N = 3$) in the different experiments treated with mock or mannan was normalized with F-HIV set as 100%. (C, D) The level of HIV-1 infection in (C) DCs ($N = 4–7$) or (D) T cells ($N = 4–9$) in the different experiments treated with mock, anti-MMR mAb, or isotype control mAb was normalized with F-HIV set as 100%. Statistical significance was tested using a two-sided paired *t*-test. $^*p < 0.05$, $^{**}p < 0.001$, $^{***}p < 0.0001$. Data are shown as mean + SEM of the indicated number of samples, each assessed in its own experiment.

e.g. cervical secretions, complement components, epithelial cell barrier, hormones, and immune cells [16]. We used a tissue explant culture system to measure the HIV-1 infection of emigrating cervical DCs and $CD4^+$ T cells after challenging the cervical explants with different forms of opsonized HIV-1 that are potentially relevant in vivo, i.e. virions covered in complement fragments or/and antibodies. Virions opsonized with complement produced higher levels of infection in the emigrating DCs than free HIV-1. In contrast, infection of emigrating $CD4^+$ T cells was decreased when the virions were opsonized. The differential effects of complement-opsonized HIV could be due to the fact that only a few T cells express CR3 [30] and the coating of HIV with iC3b affects the ability of the viral protein gp120 to interact with the CD4 receptor on the cell surface. DCs on the other hand typically express CR3 and this receptor enhances the binding and uptake of C-HIV [9] and is involved in the enhanced HIV-1 infection [21]. Blockade of CD4, and the integrins β1, β2, αM, α4, and β7, and the C-type lectin MMR resulted in significantly decreased infection of DCs. For emigrating $CD4^+$ T cells, the highest impairment of infection was seen when HIV-1 ability to bind to the CD4 receptor was neutralized but blocking the integrins β1, β2, α4, and β7 also decreased the infection. Blockade of the αM/β2- and α4-integrins inhibited infection of emigrating DCs by complement-opsonized virions more than it inhibited infection by free virions.

Within the cervix, T cells and APCs are most frequently found in the cervical transformation zone and surrounding tissues [5]. Several different types of DCs exist in the cervical mucosa including myeloid DCs, with the LCs located in the epithelial layer and DCs in the submucosa, and a small number of plasmacytoid DCs [31]. In human explant cultures, HIV-1 can rapidly reach both LCs and $CD4^+$ T cells located in the cervical epithelial layer [8], although DCs have been implicated in vaginal SIV transmission and dissemination to draining LNs in macaques [32].

We have previously demonstrated that complement opsonization of HIV-1 increased binding and uptake by both immature and mature DCs compared with free virions [9]. Furthermore, in other studies, infection of DCs, monocytes, and epithelial cells was enhanced when HIV-1 virions were complement opsonized [19, 20, 33]. This study shows for the first time that complement opsonization of HIV-1 significantly increases the infection of DCs/LCs emigrating from the cervical mucosa and decreases the infection of $CD4^+$ T cells. This was observed when HIV-1 was opsonized not only with fresh human sera but also when HIV-1 was opsonized in seminal fluid, despite the tenfold lower amount of C3 fragments in semen [33].

© 2013 The Authors. European Journal of Immunology published by WILEY-VCH Verlag GmbH & Co. KGaA Weinheim.

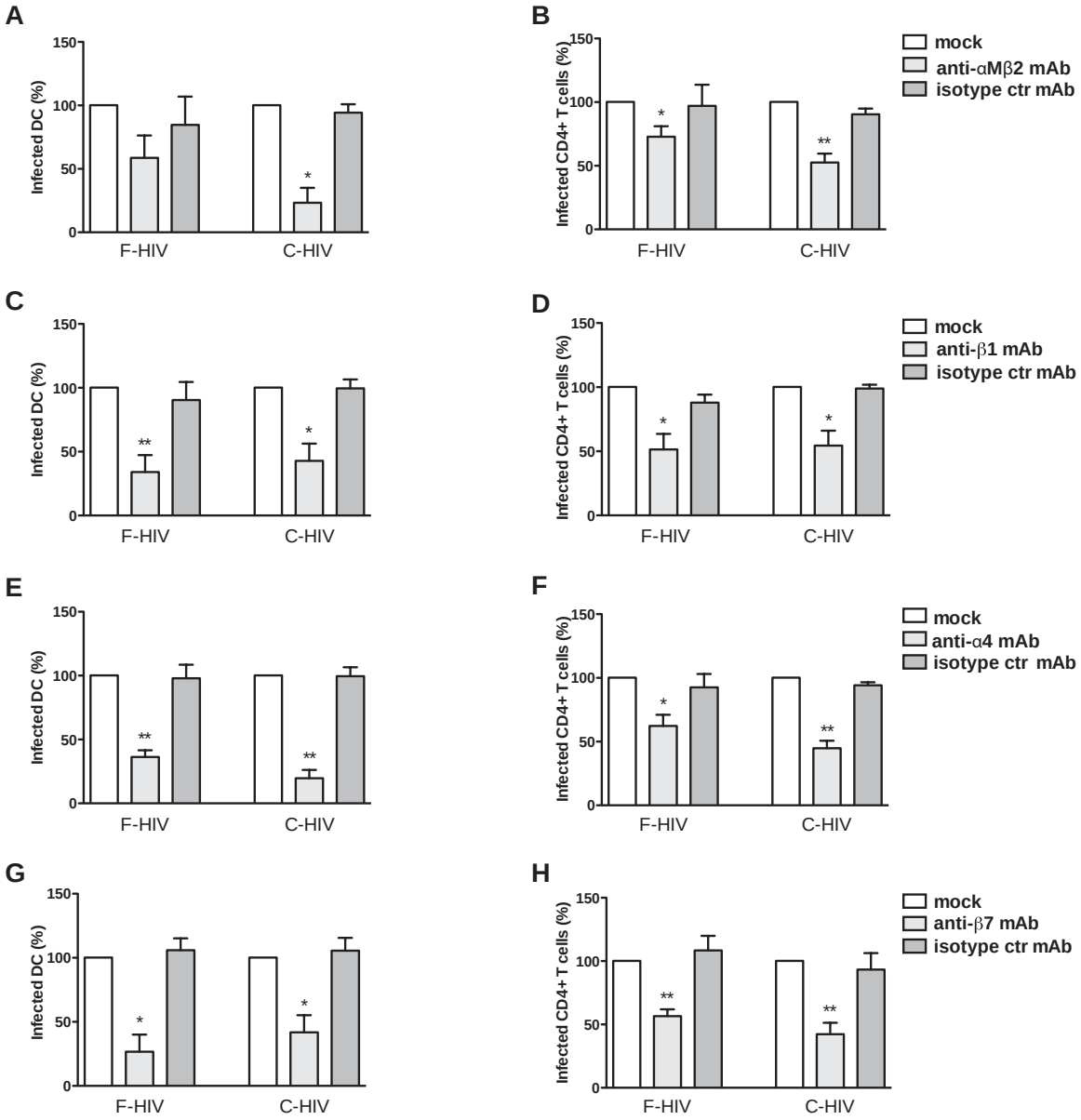

Figure 5. Blockade of integrins decreases the infection of DCs and CD4$^+$ T cells emigrating from cervical mucosa tissues. Cervical tissue biopsies were pretreated for 30 min at 37°C with mock, anti-CD11b/CD18 mAbs, anti-β1 mAb, anti-α4 mAb, anti-β7 mAb, or isotype control mAbs followed by infection with different forms of HIV-1_{BaL}, either free (F-HIV) or complement opsonized (C-HIV), by spinning the cultures for 2 h at 37°C. The tissues were washed and transferred to six-well plates and cultured for 3–6 days with mock, anti-CD11b/CD18 mAbs, anti-β1 mAb, anti-α4 mAb, anti-β7 mAb, or isotype control mAbs. The emigrating cells were harvested and stained with anti-CD1a, and anti-HIV-1 mAbs for DCs and anti-CD3, anti-CD4, and anti-HIV-1 mAbs for CD4$^+$ T cells and level of infection was assessed by flow cytometry (A, B) The level of HIV-1 infection in (A) DCs ($N = 3$) or (B) T cells ($N = 6$) in experiments treated with mock, anti-CD11b/CD18 mAbs, or isotype control mAbs was normalized with F-HIV set as 100%. (C, D) The level of HIV-1 infection in (C) DCs ($N = 5$) or (D) T cells ($N = 6$) in the different experiments treated with mock, anti-β1 mAb, or isotype control mAbs was normalized with F-HIV set as 100%. (E, F) The level of HIV-1 infection in (E) DCs ($N = 3$–4) or (F) T cells ($N = 4$) in the different experiments treated with mock, anti-α4 mAb, or isotype control mAbs was normalized with F-HIV set as 100%. (G, H) The level of HIV-1 infection in (G) DCs ($N = 3$–4) or (H) T cells ($N = 4$) in the different experiments treated with mock, anti-β7 mAb, or isotype control mAbs was normalized with F-HIV set as 100%. Statistical significance was tested using a two-sided paired *t*-test. $^*p < 0.05$, $^{**}p < 0.001$, $^{***}p < 0.0001$. Data are shown as mean + SEM of the indicated number of samples, each assessed in its own experiment.

Exposing the cervical tissue to the neutralizing mAb b12 decreased the infection of both F-HIV and C-HIV in emigrating DCs and CD4$^+$ T cells, which is consistent with prior observations on the usage of the CD4 receptor for infection of cervical mucosal DCs and T cells [24]. Neutralization of HIV-1 with b12 blocked both local infection of the cervical mucosa explants and viral dissemination of free HIV-1_{BaL} to emigrating immune cells [24], findings consistent with the observation that b12 inhibited HIV-1 replication in the vaginal mucosa in macaques when the antibody was given in high doses [34]. However, previous

© 2013 The Authors. European Journal of Immunology published by WILEY-VCH Verlag GmbH & Co. KGaA Weinheim.

studies have shown that HIV-1 can mutate to escape neutralization, potentially limiting the protective activity ability of b12 and other neutralizing antibodies. We clearly demonstrate that other receptors besides the CD4 receptor and coreceptor, i.e. integrins, can be of importance for the interactions between virus and potential target cells and for infection of $CD4^+$ T cells in the cervical mucosa, possibly by aiding viral attachment to the cell surface and/or facilitating viral synapse formation [26] and dissemination of HIV-1.

MMR is expressed by DCs located in cervical tissues and on DCs emigrating from cervical explants [24]. HIV-1 uptake via MMR into macrophages and via Langerin into LCs can guide the virions away from the route leading to productive infection [35, 36]. We established that C-type lectins were involved in events leading to infection of emigrating DCs as mannan and anti-MMR mAb decreased the infection. Although the effect of mannan on HIV-1 infection of cervical tissue explants and the ability of emigrating cells to cross-infect target cells has been shown previously [24], our data show direct infection of emigrating DCs from the cervical explants. However, no protection against SIV infection was seen for mannan in a macaque model [37]. Consequently, blocking of C-type lectins may not give the desired outcome as the viral interactions with these receptors are complex and may affect infection in different ways depending on the cell type.

Integrins bind to various cell surface and extracellular proteins to mediate cell-to-cell matrix adhesion and are involved in multiple important biological functions. Many viruses have been shown to use integrins to interact with host cells in order to facilitate or mediate infection [25]. For example, the integrin β1 (CD29) is expressed by almost all cells and has been shown to be involved in rotavirus internalization and reovirus cell entry and infection [25]. In the cervical mucosa, β1-integrin is expressed on the basal membrane of epithelial cells and on immune cells, with strongest expression at the basal membrane layer [38]. A previous study showed that blocking β1-integrins on cervical mucosa epithelia diminished attachment of HIV-1 virions [38]. The blocking of HIV-1 usage of this receptor in the cervical mucosa decreased infection of both DCs and T cells emigrating out of the tissues and this could be an effect of direct interaction of HIV-1 with β1-integrins expressed on the immune cells or an indirect effect of viral binding to epithelial cells and the basal membrane expressing integrin β1.

α4β7 is the submucosal homing receptor expressed by T cells and DCs located in the gut and genital mucosa [26]. HIV-1 gp120 binds to an activated form of α4β7, and on T cells this binding leads to activation of LFA-1, facilitating efficient spread of virus to other cells via virological synapses [26]. A variety of observations suggest that interactions between gp120 and α4β7 may play a role in mucosal transmission and/or early mucosal replication of HIV-1 [15, 39]. Of note, blocking α4β7-integrin by intravenous administration during acute SIV infection leads to decreased viral load in infected rhesus macaques [40]. Blocking of α4- and β7-integrins significantly diminished HIV-1 infection of DCs and T cells emigrating from cervical tissue explants, possibly reflecting involvement of α4β7 in complex with CD4 on the DCs and T cells in direct infection and/or the formation of virological synapses and dissemination of virions between immune cells.

Following vaginal SIV mucosal challenge of macaques, small populations of infected cells are established, which undergo a local expansion before disseminating to establish systemic infection [13]. These small founder populations must be sustained at a basic reproductive ratio (R_0) > 1 to give rise to systemic infection, otherwise the infection will be aborted [13, 14]. The inhibition seen in infection of mucosal $CD4^+$ T cells and DCs when blocking integrins may substantially decrease the founder populations in the cervical mucosa. These findings suggest that compounds targeting integrins might be successful in preventing the establishment of HIV-1 infection. However, there could be negative effects of blocking integrins on the mucosa and immune cells' ability to form immunological synapse and this needs to be assessed in animal models as these factors play important roles in modulating immune responses. Recent trials with microbicides and vaccines have shown some ability to prevent HIV-1 transmission but the efficacy has been low. A deeper understanding of how HIV-1 interacts with the immune cells in the cervicovaginal mucosa is necessary to prevent transmission and HIV infection in the future. New molecules to target for the use in new vaccines and microbicides are of great interest and our results suggest that integrins, such as α4 and β7, may be promising microbicide candidates.

Materials and methods

Cervical tissue samples Ethics statement

This study was approved by Linköping Ethical Review Board (Ethical permit EPN M206-06) and informed consent of all participating subjects was obtained. Normal cervical tissue samples ($N = 42$) were obtained mainly from women who underwent prolapse surgery and from a few women who underwent hysterectomy for benign conditions comprising menstrual bleeding disorders and myomas at the Gynecology Clinic in Linköping, Sweden. All women had normal Pap smears prior to surgery and showed cytologically and clinically no signs of infection/inflammation at surgery.

Virus propagation

$HIV\text{-}1_{BaL}$/SUPT1-CCR5 CL.30 (Lot #P4143: Biological Products Core/AIDS and Cancer Virus Program, SAIC-Frederick, Inc., NCI Frederick) was produced using chronically infected cultures of ACVP/BPC cell line (No. 204). Virus was purified by continuous flow centrifugation for 30 min after sample loading. Virus containing fractions were pooled and diluted to <20% sucrose, and virus pelleted at approximately 100 000 × g for 1 h. The virus pellet was resuspended and aliquots were frozen in liquid N_2 vapor. All virus preparations were assayed for infectivity.

© 2013 The Authors. European Journal of Immunology published by WILEY-VCH Verlag GmbH & Co. KGaA Weinheim.

Opsonization of HIV-1

We used different preparations of HIV-1; F-HIV, HIV-1 opsonized with complement (C-HIV), HIV opsonized with a mixture of unspecific and HIV-1-specific IgG (IgG-HIV), or HIV-1 opsonized with a combination of both complement and IgG (C-IgG-HIV). C-HIV was obtained by incubation of HIV-1_{BaL} (30 μg/mL p24 equivalent) with an equal volume of human serum (HS) or seminal plasma containing 25% Veronal buffer [33]. To obtain C-IgG-HIV, 0.20 μg/mL neutralizing HIV-specific IgG (SMI, Stockholm, Sweden) and 20 μg/mL gamma globulin (Pharmacia, Stockholm, Sweden) were added besides the HS containing Veronal buffer, whereas IgG-HIV was obtained by adding only the antibodies. F-HIV was treated with media alone. All groups were incubated for 1 h at 37°C. As a negative control, human sera were heat-inactivated for 30 min at 56°C, incubated with HIV-1 and prepared under the same conditions as the other groups.

Cervical tissue samples and virus inoculations

The cervical tissues were kept on ice and processed within 30 min after resection. The epithelial layer and lamina propria were separated from underlying stroma and the tissue explants (8 mm^2) were placed in cell culturing plates containing RPMI 1640, 20 μg/mL Gentamicin, 10 mM HEPES (Fisher Scientific, Gothenburg, Sweden), and 5% pooled HS (Fisher Scientific). Cervical explants were preincubated for 30 min at 37°C for mock treatment or with different inhibitors. The following inhibitors were used: 10 mg/mL mannan, 10 μM AZT (SIGMA-Aldrich), 5 mg/mL EDTA (SIGMA-Aldrich), mAb against the CD4 binding site on gp120 (b12, a kind gift from Professor D. Burton), anti-αM integrin mAb (CD11b: ICRF44, Ancell Corp., Bayport, NY, USA), anti-αM integrin mAb (KIM75), and anti-β2 integrin mAb (CD18: clone 6.5) (kind gifts from M. Robinson, Celltech, UCB group), anti-MMR mAb (CD206: clone 15-2, Biosite), anti-α4 integrin mAb (clone P1H4, Abcam), anti-β7 integrin mAb (clone F1B504, Biosite), and anti-β1 integrin (CD29) (clone JB1A, Millipore); matching isotype controls were used.

Tissue samples were challenged with HIV-1_{BaL} (250 ng/mL), spinocculated for 2 hours at 37°C, washed four times, transferred to six-well tissue culture plates, and cultured. After 3–6 days the emigrating cervical cells were collected and stained for flow cytometry. The infected cervical explants were fixed in 4% paraformaldehyde (PFA) and stored for later immunohistochemisty and/or immunofluorescence staining. To compare results obtained from separate experiments, using cervical explants derived from different donors, the F-HIV and C-HIV infection of emigrating DCs and $CD4^+$ T cells in absence of inhibitors was normalized to 100% for each experiment.

Flow cytometry

Emigrated cells were harvested on day 3–6 from cervix cultures and stained with the following anti-human mAbs; CD4-allophycocyanin (BD Biosciences), CD3-PerCP (BD Biosciences), and CD1a-PE (BD Biosciences). For detection of HIV-1, the cells were fixed in 4% PFA, permeabilized in 0.2% saponin, and incubated with the anti-HIV-1 mAb (KC57, clone FH190-1-1) and the corresponding isotype control (BD PharMingen, SanDiego, CA, USA) for 45 min. The stained cells were assessed by flow cytometry (FACS Calibur) and acquired data were analyzed by FlowJo (Treestar, Ashland, OR, USA). In addition, the emigrating immune cells were stained with mAbs against Langerin (CloneD-CGM4/122D5 Biosite), MMR, DC-SIGN, α4, αM, β1, β7, α4, or β7 followed by sAb conjugated with FITC for 1 h followed by CD1a-PE or CD3-PerCP staining.

Immunofluorescence and confocal microscopy

Cryopreserved cervical tissue explants were sectioned and the sections were fixed in 4% PFA, quenched, and stained with the following mAbs: CD1a (HI149, BioLegend, San Diego, CA, USA), Langerin, MMR, α4, β7, α4β7, αVβ5, and β1. The slides were incubated with antibodies over night at 4°C, washed three times, and stained with a secondary mAb (Rhodamine Red-X conjugated donkey anti-mouse IgG, Jackson ImmunoResearch, Baltimore, PA, USA) for 1 h at room temperature. The sections were then washed and mounted using mounting medium containing DAPI (Vector Laboratories, Burlingame, CA, USA). The samples were analyzed using an LSM 510 META confocal microscope (Carl Zeiss AB, Stockholm, Sweden) and LSM 510 software.

Statistical analysis

GraphPad Prism 5 (GraphPad Software, La Jolla, CA, USA) was used for statistical analysis. Results were tested for statistical significance using a two-sided paired *t*-test and *p*-values less than 0.05 were considered statistically significant. In all figures, *N* denotes the number of times each experiment was replicated, using cervical explants derived from different women.

Acknowledgements: We thank the Biological Products Core of the AIDS and Cancer Virus Program, SAIC Frederick, Inc., National Cancer Institute, Frederick, MD, USA for providing infectious and AT-2 inactivated HIV-1. This work was supported by grants through: Marie Larsson: AI52731, The Swedish Research Council, The Swedish, Physicians Against AIDS research Foundation, The Swedish International Development Cooperation Agency; SIDA SARC, VINNMER for Vinnova, Linköping University hospital research Fund, C ALF, and The Swedish Society of Medicine, and in part with federal funds from the National Cancer Institute, National Institutes of Health, under contract HHSN261200800001E (J.L.).

© 2013 The Authors. European Journal of Immunology published by WILEY-VCH Verlag GmbH & Co. KGaA Weinheim.

Conflict of interest: The authors declare no financial or commercial conflict of interest.

References

1 **Merbah, M., Introini, A., Fitzgerald, W., Grivel, J. C., Lisco, A., Vanpouille, C. and Margolis, L.**, Cervico-vaginal tissue ex vivo as a model to study early events in HIV-1 infection. *Am. J. Reprod. Immunol.* 2011. **65**: 268–278.

2 **Hu, J., Gardner, M. B. and Miller, C. J.**, Simian immunodeficiency virus rapidly penetrates the cervicovaginal mucosa after intravaginal inoculation and infects intraepithelial dendritic cells. *J. Virol.* 2000. **74**: 6087–6095.

3 **Brenchley, J. M. and Douek, D. C.**, HIV infection and the gastrointestinal immune system. *Mucosal Immunol.* 2008. **1**: 23–30.

4 **Jordan, J., Singer, A., Jones, H. and Shafi, M. (Eds.)** *The Cervix*, (2nd ed). Wiley Blackwell Press, 2006.

5 **Pudney, J., Quayle, A. J. and Anderson, D. J.**, Immunological microenvironments in the human vagina and cervix: mediators of cellular immunity are concentrated in the cervical transformation zone. *Biol. Reprod.* 2005. **73**: 1253–1263.

6 **Haase, A. T.**, Early events in sexual transmission of HIV and SIV and opportunities for interventions. *Annu. Rev. Med.* 2011. **62**: 127–139.

7 **de Jong, M. A., de Witte, L., Oudhoff, M. J., Gringhuis, S. I., Gallay, P. and Geijtenbeek, T. B.**, TNF-alpha and TLR agonists increase susceptibility to HIV-1 transmission by human Langerhans cells ex vivo. *J. Clin. Invest.* 2008. **118**: 3440–3452.

8 **Hladik, F., Sakchalathorn, P., Ballweber, L., Lentz, G., Fialkow, M., Eschenbach, D. and McElrath, M. J.**, Initial events in establishing vaginal entry and infection by human immunodeficiency virus type-1. *Immunity* 2007. **26**: 257–270.

9 **Tjomsland, V., Ellegard, R., Che, K., Hinkula, J., Lifson, J. D. and Larsson, M.**, Complement opsonization of HIV-1 enhances the uptake by dendritic cells and involves the endocytic lectin and integrin receptor families. *PLoS One* 2011. **6**: e23542.

10 **Turville, S. G., Arthos, J., Donald, K. M., Lynch, G., Naif, H., Clark, G., Hart, D. et al.**, HIV gp120 receptors on human dendritic cells. *Blood* 2001. **98**: 2482–2488.

11 **de Witte, L., Bobardt, M., Chatterji, U., Degeest, G., David, G., Geijtenbeek, T. B. and Gallay, P.**, Syndecan-3 is a dendritic cell-specific attachment receptor for HIV-1. *Proc. Natl. Acad. Sci. USA* 2007. **104**: 19464–19469.

12 **Piguet, V. and Steinman, R. M.**, The interaction of HIV with dendritic cells: outcomes and pathways. *Trends Immunol.* 2007. **28**: 503–510.

13 **Miller, C. J., Li, Q., Abel, K., Kim, E. Y., Ma, Z. M., Wietgrefe, S., La Franco-Scheuch, L. et al.**, Propagation and dissemination of infection after vaginal transmission of simian immunodeficiency virus. *J. Virol.* 2005. **79**: 9217–9227.

14 **Zhang, Z., Schuler, T., Zupancic, M., Wietgrefe, S., Staskus, K. A., Reimann, K. A., Reinhart, T. A. et al.**, Sexual transmission and propagation of SIV and HIV in resting and activated CD4+ T cells. *Science* 1999. **286**: 1353–1357.

15 **Nawaz, F., Cicala, C., Van Ryk, D., Block, K. E., Jelicic, K., McNally, J. P., Ogundare, O. et al.**, The genotype of early-transmitting HIV gp120s promotes alpha (4) beta(7)-reactivity, revealing alpha (4) beta(7) +/CD4 +T cells as key targets in mucosal transmission. *PLoS Pathog.* 2011. **7**: e1001301.

16 **Haase, A. T.**, Targeting early infection to prevent HIV-1 mucosal transmission. *Nature* 2010. **464**: 217–223.

17 **Stoiber, H., Pruenster, M., Ammann, C. G. and Dierich, M. P.**, Complement-opsonized HIV: the free rider on its way to infection. *Mol. Immunol.* 2005. **42**: 153–160.

18 **Stoiber, H., Banki, Z., Wilflingseder, D. and Dierich, M. P.**, Complement-HIV interactions during all steps of viral pathogenesis. *Vaccine* 2008. **26**: 3046–3054.

19 **Thieblemont, N., Delibrias, C., Fischer, E., Weiss, L., Kazatchkine, M. D. and Haeffner-Cavaillon, N.**, Complement enhancement of HIV infection is mediated by complement receptors. *Immunopharmacology* 1993. **25**: 87–93.

20 **Bajtay, Z., Speth, C., Erdei, A. and Dierich, M. P.**, Cutting edge: productive HIV-1 infection of dendritic cells via complement receptor type 3 (CR3, CD11b/CD18). *J. Immunol.* 2004. **173**: 4775–4778.

21 **Bouhlal, H., Chomont, N., Requena, M., Nasreddine, N., Saidi, H., Legoff, J., Kazatchkine, M. D. et al.**, Opsonization of HIV with complement enhances infection of dendritic cells and viral transfer to CD4 T cells in a CR3 and DC-SIGN-dependent manner. *J. Immunol.* 2007. **178**: 1086–1095.

22 **Wilflingseder, D., Banki, Z., Garcia, E., Pruenster, M., Pfister, G., Muellauer, B., Nikolic, D. S. et al.**, IgG opsonization of HIV impedes provirus formation in and infection of dendritic cells and subsequent long-term transfer to T cells. *J. Immunol.* 2007. **178**: 7840–7848.

23 **Greenhead, P., Hayes, P., Watts, P. S., Laing, K. G., Griffin, G. E. and Shattock, R. J.**, Parameters of human immunodeficiency virus infection of human cervical tissue and inhibition by vaginal virucides. *J. Virol.* 2000. **74**: 5577–5586.

24 **Hu, Q., Frank, I., Williams, V., Santos, J. J., Watts, P., Griffin, G. E., Moore, J. P. et al.**, Blockade of attachment and fusion receptors inhibits HIV-1 infection of human cervical tissue. *J. Exp. Med.* 2004. **199**: 1065–1075.

25 **Stewart, P. L. and Nemerow, G. R.**, Cell integrins: commonly used receptors for diverse viral pathogens. *Trends Microbiol.* 2007. **15**: 500–507.

26 **Arthos, J., Cicala, C., Martinelli, E., Macleod, K., Van Ryk, D., Wei, D., Xiao, Z. et al.**, HIV-1 envelope protein binds to and signals through integrin alpha4beta7, the gut mucosal homing receptor for peripheral T cells. *Nat. Immunol.* 2008. **9**: 301–309.

27 **Rivera-Nieves, J., Olson, T., Bamias, G., Bruce, A., Solga, M., Knight, R. F., Hoang, S. et al.**, L-selectin, alpha 4 beta 1, and alpha 4 beta 7 integrins participate in CD4+ T cell recruitment to chronically inflamed small intestine. *J. Immunol.* 2005. **174**: 2343–2352.

28 **Danussi, C., Petrucco, A., Wassermann, B., Pivetta, E., Modica, T. M., Del Bel Belluz, L., Colombatti, A. et al.**, EMILIN1-alpha4/alpha9 integrin interaction inhibits dermal fibroblast and keratinocyte proliferation. *J. Cell Biol.* **195**: 131–145.

29 **Garmy-Susini, B., Jin, H., Zhu, Y., Sung, R. J., Hwang, R. and Varner, J.**, Integrin alpha4beta1-VCAM-1-mediated adhesion between endothelial and mural cells is required for blood vessel maturation. *J. Clin. Invest.* 2005. **115**: 1542–1551.

30 **Muto, S., Vetvicka, V. and Ross, G. D.**, CR3 (CD11b/CD18) expressed by cytotoxic T cells and natural killer cells is upregulated in a manner similar to neutrophil CR3 following stimulation with various activating agents. *J. Clin. Immunol.* 1993. **13**: 175–184.

31 **Hirbod, T., Kaldensjo, T., Lopalco, L., Klareskog, E., Andersson, S., Uberti-Foppa, C., Ferrari, D. et al.**, Abundant and superficial expression of C-type lectin receptors in ectocervix of women at risk of HIV infection. *J. Acquir. Immune. Defic. Syndr.* 2009. **51**: 239–247.

32 **Miller, C. J. and Lu, F. X.**, Anti-HIV and -SIV immunity in the vagina. *Int. Rev. Immunol.* 2003. **22**: 65–76.

33 **Bouhlal, H., Chomont, N., Haeffner-Cavaillon, N., Kazatchkine, M. D., Belec, L. and Hocini, H.**, Opsonization of HIV-1 by semen complement

© 2013 The Authors. European Journal of Immunology published by WILEY-VCH Verlag GmbH & Co. KGaA Weinheim.

enhances infection of human epithelial cells. *J. Immunol.* 2002. **169**: 3301–3306.

34 **Hessell, A. J., Rakasz, E. G., Poignard, P., Hangartner, L., Landucci, G., Forthal, D. N., Koff, W. C. et al.**, Broadly neutralizing human anti-HIV antibody 2G12 is effective in protection against mucosal SHIV challenge even at low serum neutralizing titers. *PLoS Pathog.* 2009. **5**: e1000433.

35 **de Witte, L., Nabatov, A., Pion, M., Fluitsma, D., de Jong, M. A., de Gruijl, T., Piguet, V. et al.**, Langerin is a natural barrier to HIV-1 transmission by Langerhans cells. *Nat. Med.* 2007. **13**: 367–371.

36 **Trujillo, J. R., Rogers, R., Molina, R. M., Dangond, F., McLane, M. F., Essex, M. and Brain, J. D.**, Noninfectious entry of HIV-1 into peripheral and brain macrophages mediated by the mannose receptor. *Proc. Natl. Acad. Sci. USA* 2007. **104**: 5097–5102.

37 **Veazey, R. S., Klasse, P. J., Schader, S. M., Hu, Q., Ketas, T. J., Lu, M., Marx, P. A. et al.**, Protection of macaques from vaginal SHIV challenge by vaginally delivered inhibitors of virus-cell fusion. *Nature* 2005. **438**: 99–102.

38 **Maher, D., Wu, X., Schacker, T., Horbul, J. and Southern, P.**, HIV binding, penetration, and primary infection in human cervicovaginal tissue. *Proc. Natl. Acad. Sci. USA* 2005. **102**: 11504–11509.

39 **Cicala, C., Martinelli, E., McNally, J. P., Goode, D. J., Gopaul, R., Hiatt, J., Jelicic, K. et al.**, The integrin alpha4beta7 forms a complex with cell-surface CD4 and defines a T-cell subset that is highly susceptible to infection by HIV-1. *Proc. Natl. Acad. Sci. USA* 2009. **106**: 20877–20882.

40 **Ansari, A. A., Reimann, K. A., Mayne, A. E., Takahashi, Y., Stephenson, S. T., Wang, R., Wang, X. et al.**, Blocking of alpha4beta7 gut-homing integrin during acute infection leads to decreased plasma and gastrointestinal tissue viral loads in simian immunodeficiency virus-infected rhesus macaques. *J. Immunol.* 2011. **186**: 1044–1059.

Abbreviations: **AZT**: azidothymidine · **C-HIV**: complement opsonized HIV-1 · **F-HIV**: free HIV-1 · **HS**: human serum · **LC**: Langerhans cell · **LP**: lamina propria · **PFA**: paraformaldehyde

Full correspondence: Prof. Marie Larsson, Molecular Virology, Department of Clinical and Experimental Medicine, Linköping University, 581 85 Linköping, Sweden
Fax: +46-10-1031375
e-mail: marie.larsson@liu.se

Received: 14/12/2012
Revised: 31/3/2013
Accepted: 14/5/2013
Accepted article online: 18/5/2013

© 2013 The Authors. European Journal of Immunology published by WILEY-VCH Verlag GmbH & Co. KGaA Weinheim.