

Neutralizing monoclonal antibodies to human immunodeficiency virus type 1 do not inhibit viral transcytosis through mucosal epithelial cells

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Abstract

HIV-1 transcytosis has been proposed as a potential mechanism allowing the virus to cross the epithelium during mucosal transmission. Epitopes of the HIV-1 envelope involved in this process have not been identified yet. Here, we assessed a large panel of HIV neutralizing antibodies recognizing well-characterized epitopes of the HIV-1 envelope for their ability to block HIV-1 transcytosis across a confluent epithelial monolayer.

We found that all of the 13 HIV-1-specific monoclonal antibodies tested in the present study, including the three broadly neutralizing antibodies 2F5, 2G12 and IgG1b12, lacked the ability to inhibit transcytosis of cell-free and cell-associated R5- as X4-tropic HIV-1 across a tight and polarized monolayer of HEC-1 epithelial cells. In contrast, anti-gp160 polyclonal antibodies purified from serum or breast milk of HIV-1-infected individuals potently inhibited HIV-1 transcytosis. Furthermore, polymeric S-IgA exhibited similar ability to inhibit transcytosis compared to IgG despite their lower anti-gp160 specific activity. Together, these results demonstrate that the major neutralizing envelope epitopes of HIV-1 are not involved in HIV-1 transcytosis, and suggest that surface agglutination of virus particles may participate to the blocking effect observed with both polyclonal and polymeric anti-gp160 immunoglobulins.

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Introduction

Human immunodeficiency virus type 1 (HIV-1) is primarily transmitted via mucosae (Van de Perre, 1999; Yeaman et al., 1998). Several in vitro models have been developed to study the mechanisms involved in the transmucosal passage of the virus (Bomsel, 1997; Shattock et al., 2000). Transcytosis of HIV-1 through a tight monolayer of epithelial cells, such as human endometrial carcinoma (HEC-1) cells, has been used to evaluate

the initial interactions between cell-free and cell-associated virus and mucosal epithelial cells, as well as the intracellular transport of HIV from the apical to the basolateral pole of the monolayer (Bomsel, 1997). Both cell-free and cell-associated viruses are able to cross transformed and primary epithelial cells monolayers (Kage et al., 1998; Meng et al., 2002). Transcytosis of HIV-1 does not occur at 4 °C (Hocini et al., 2001) and is affected by inhibitors of the microtubular activity like colchicine, indicating that this phenomena is an active process. The early steps of the transcytosis process involve specific surface molecules of the virus and epithelial cells. Thus, galactosylceramide (GalCer), one of the alternative receptors for HIV-1 on CD4-negative epithelial cells (Fantini et al., 1993; Yahi et al., 1992), interacts with a conserved epitope on the DIII determinant of gp41 in the initiation of transcytosis (Alfsen and

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Table 1

Neutralizing activity of 13 monoclonal antibodies directed to well-defined epitopes of the HIV-1 envelope, on peripheral blood lymphocytes infected by various strains of HIV-1^a

				HIV-1 strains				
				Ba-L	JR-CSF	NDK	VN44	Lai
				TCLA/R5 ^c	Primary/R5	Primary/X4	Primary/X4	TCLA/X4
Monoclonal antibodies to HIV-1	2F5	Human, IgG3 κ ^b	gp41 ^d	60.8 (7.4) ^a	60.3 (3.8)	39.3 (12.2)	42.3 (11.2)	96.2 (4.7)
	2G12	Human, IgG1 κ	gp120 (V3–V4)	74.1 (7.9)	90.3 (9.1)	59.7 (19.1)	48.0 (14.9)	51.6 (5.2)
	IgG1b12	Human, IgG1 κ	gp120 (CD4 BS)	98.6 (2.6)	97.0 (4.5)	65.6 (14.7)	77.4 (9.9)	98.5 (0.3)
	17b	Human	gp120 (CD4i)	43.4 (8.8)	41.2 (8.2)	–0.9 (1.0)	47.6 (6.5)	91.2 (2.1)
	48d	Human, IgG1 κ	gp120 (CD4i)	44.0 (10.3)	58.4 (14.1)	–35.0 (0.6)	–36.0 (5.1)	8.5 (11.2)
	IIIB-V3-21	Mouse, IgG1	gp120	20.3 (6.5)	–8.6 (14.5)	15.1 (6.2)	–1.0 (3.5)	17.8 (4.8)
	F105	Human, IgG1 κ	gp120 (CD4 BS)	40.4 (8.1)	28.7 (6.6)	17.9 (8.8)	49.5 (6.5)	–65.9 (17.4)
	5F3	Human, IgG1 κ	gp41	38.4 (19.0)	29.5 (39.0)	8.2 (7.3)	9.8 (0.5)	54.1 (0.7)
	F240	Human, IgG1 κ	gp41	48.6 (11.4)	40.0 (3.0)	13.3 (2.5)	8.7 (2.3)	60.5 (10.8)
	1577	Mouse	gp41	19.5 (5.5)	33.0 (15.3)	5.1 (3.5)	22.2 (0.8)	34.2 (0.3)
	F425B4a1	Human	gp120 (V3)	33.5 (3.6)	38.7 (2.9)	16.9 (7.6)	16.4 (18.5)	33.8 (2.7)
	F425A1g8	Human, IgG1 λ	gp120 (CD4i)	36.9 (3.6)	43.3 (6.1)	17.3 (11.1)	20.6 (13.5)	50.4 (9.5)
	F425B4e8	Human, IgG2 κ	gp120 (V3)	47.0 (9.4)	42.6 (3.9)	49.6 (14.4)	35.8 (1.0)	18.2 (1.3)

^a Results indicate the percentages of inhibition provided by 20 μ g/ml of monoclonal antibodies, calculated by reference to the control experiment with irrelevant IgG (no inhibition). Neutralization was quantified by p24 antigen release. Standard deviations are in brackets. Results of monoclonal antibodies giving significant inhibition are indicated in bold.

^b Species and isotype of antibodies.

^c Phenotypic characteristics of viral strains [primary or TCLA (T Cell Line Adapted) strains; R5- or X4-tropism].

^d Main recognized epitopes on HIV-1 surface. CD4 BS: CD4 binding site; CD4i: CD4 induced.

Bomsel, 2002; Alfsen et al., 2001). Other virus-encoded surface epitopes are also required for the early steps of transcytosis, as demonstrated by the ability of polyclonal antibodies to gp160 to block HIV-1 transcytosis (Hocini et al., 2001). The latter observation has allowed to use transcytosis assays as a mean to functionally assess HIV-specific antibodies in the saliva (Devito et al., 2000), cervicovaginal secretions (Belec et al., 2001; Devito et al., 2000) and breast milk (Alfsen et al., 2001; Becquart et al., 1999, 2000) in HIV-1-infected individuals. Using this assay, HIV-specific antibodies purified from cervicovaginal fluids in a minority of highly exposed, persistently HIV-seronegative female sex workers have been shown to inhibit transcytosis, suggesting that these antibodies play a role in the protection against sexual acquisition of the virus in vivo (Belec et al., 2001; Devito et al., 2000).

In the present study, we have investigated the inhibitory activity on transcytosis of a large panel of monoclonal antibodies directed to well-defined epitopes of the HIV-1 envelope, including the 3 broadly neutralizing antibodies 2F5, 2G12 and IgG1b12, to investigate whether target epitopes for neutralizing antibodies on HIV-1 are also involved in inhibition of transcytosis. Our results indicate that transcytosis of HIV-1 through epithelial cells involves distinct molecular mechanisms than those occurring during infection of peripheral blood mononuclear cells.

Results

Neutralization experiments

We used a single dose of antibody to assess the neutralizing properties of monoclonal antibodies towards infection of PBLs

with various strains of HIV-1. Virus was pre-incubated with monoclonal antibody at a final concentration of 20.0 μ g/ml for 1 h at 37 °C. A reduction in infectivity of at least 33% was considered as significant. Under these conditions, the highest neutralization titers were obtained with monoclonal antibodies 2F5, 2G12 and IgG1b12 (Table 1). Antibodies directed against the CD4i epitope (17b and 48d) showed higher neutralizing activities against R5- than against X4-strains. HIV-1_{Lai} was efficiently neutralized (reduction in infectivity superior to 90%) by three monoclonal antibodies IgG1b12, 2F5 and 17b, whereas HIV-1_{NDK} and HIV-1_{VN44}, two primary X4-strains, were relatively resistant to neutralization, with IgG1b12 exhibiting the highest neutralizing activities of 65.6% and 77.4%, respectively.

Transcytosis of HIV-1

We measured the relative ability of six HIV-1 strains as free virus and of four HIV-1 strains as cell-associated virus, to cross a tight monolayer of HEC-1 cells. Transcytosis was assessed by p24 antigen quantification in the basal chamber after 4 h, 24 h and 48 h. Transcytosis of cell-free virus at 4h ranged from 0.05% for HIV-1_{Ba-L} to 0.49% for HIV-1_{Lai} (Fig. 1). After 48 h, this percentage reached 2.9% for HIV-1_{Lai}. No transcytosis was observed with cell-free HIV-1_{Bru Δ env}. All viral strains tested for transcytosis efficiently replicated in PBMC, with the exception of HIV-1_{Bru Δ env} (data not shown).

Transcytosis of cell-associated virus was assessed by measuring p24 antigen concentrations in the apical (neo-produced virions) and basal (transcytosed virions) chambers after 4 h and 24 h. Transcytosis of cell-associated virus ranged between 0.35% for HIV-1_{VN44} and 0.75% for HIV-1_{Lai} at 4 h (Fig. 2).

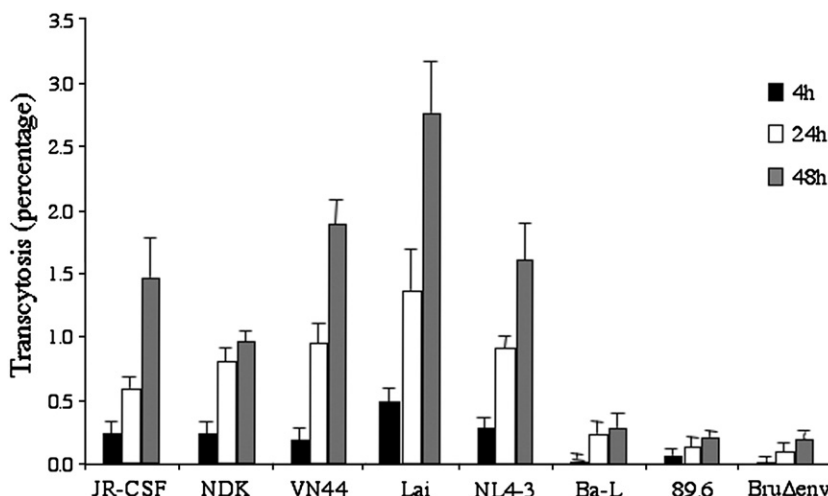


Fig. 1. Transcytosis efficiency through HEC-1 cells of eight strains of HIV-1 in the form of cell-free virus at 4 h, 24 h and 48 h. Results are expressed as percentage of virus recovered in the basal chamber as compared to the amount of virus deposited in the apical chamber (5 ng). The data are expressed as means $\pm$ standard deviation obtained in four independent experiments.

Effects of monoclonal antibodies on transcytosis of HIV-1

Each monoclonal antibody from our panel was tested for its ability to block HIV-1 transcytosis. Cell-free virus was pre-incubated with monoclonal antibodies (up to 50 μ g/ml) or with purified polyclonal anti-gp160 antibodies (positive control), and deposited on the apical surface of HEC-1 cells. Four viral strains capable of transcytosis as cell-free or cell-associated virus (HIV-1_{JR-CSF}, HIV-1_{NDK}, HIV-1_{VN44} and HIV-1_{Lai}) were tested in combination with each monoclonal antibody.

None of tested monoclonal antibodies inhibited transcytosis of cell-free HIV-1 (Fig. 3). Several antibodies increased the passage of cell-free HIV-1 through HEC-1 cells. As depicted in Fig. 3, F105 (which recognizes the binding site for HIV on CD4) significantly increased transcytosis of HIV-1_{JR-CSF} and HIV-1_{VN44} (–53.8% and –27.6%, respectively). Moreover, the mixture of the 13 monoclonal antibodies (50 μ g/ml each) did not inhibit HIV-1 transcytosis. Transcytosis of all viral strains tested was however blocked by polyclonal anti-gp160 antibodies.

In order to assess whether surface agglutination of virus particles by HIV-specific antibodies may be important to observe HIV transcytosis inhibition, we incubated during 15 min at room temperature the mixture of the 11 human monoclonal antibodies (10 μ l at 50 μ g/ml each) with cell-free HIV-1_{JR-CSF}, and after anti-human γ chain (10 μ l at 2.5 mg/ml) was added before the transcytosis assay. Polyclonal anti-gp160 was used as control. The transcytosis was then partially inhibited by 45.2% when the mixture of monoclonal antibodies and HIV-1_{JR-CSF} was incubated with polyclonal anti-human γ chain, whereas a 90%-inhibition was observed with polyclonal anti-gp160 antibody. This observation clearly confirms that agglutination of viral particles at the apical surface of the HEC-1 monolayer may be critical to obtain HIV transcytosis inhibition by HIV-specific antibodies.

Similar experiments were further carried out with cell-associated HIV-1 (Fig. 4). In such experiments, cells were not

pre-incubated with monoclonal antibodies, but both antibodies and cells were deposited on the apical surface of HEC-1 cells at the same time, in order to minimize the amount of neo-produced cell-free viral particles. As previously observed with cell-free virus, all monoclonal antibodies tested as well as the mixture of the 13 monoclonal antibodies did not inhibit transcytosis at 4 h. The concentration of p24 antigen in the basal chamber was lower at 24 h and 48 h due to the reduction of viral production in the apical compartment, rather than to inhibition of transcytosis.

Purified polyclonal antibodies to gp160 significantly inhibited the transcytosis process. Inhibition of transcytosis of cell-associated virus by polyclonal antibodies to gp160 was always lesser than inhibition of transcytosis of the corresponding virus

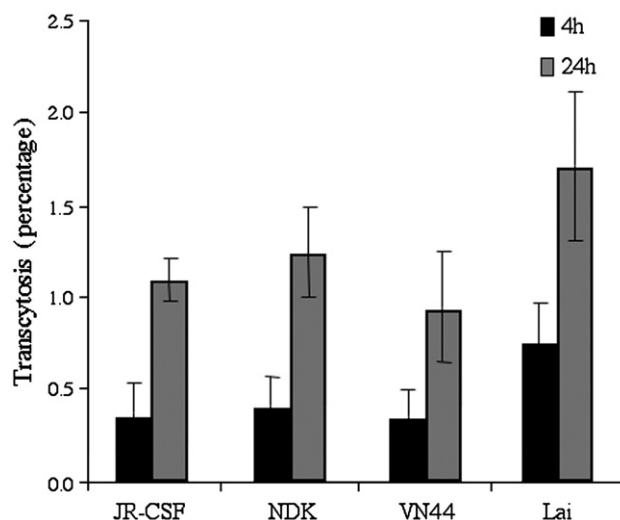


Fig. 2. Transcytosis of four HIV-1 strains in the form of cell-associated virus at 4 h and 24 h. Freshly isolated PBMC from seronegative donors were infected with the indicated virus and deposited in the apical chamber. Virus was quantified by p24 antigen ELISA. Results were expressed as percentage of virus recovered in the basal chamber as compared with the amount of virus produced in the apical chamber. The data are expressed as means $\pm$ standard deviation obtained in four independent experiments.

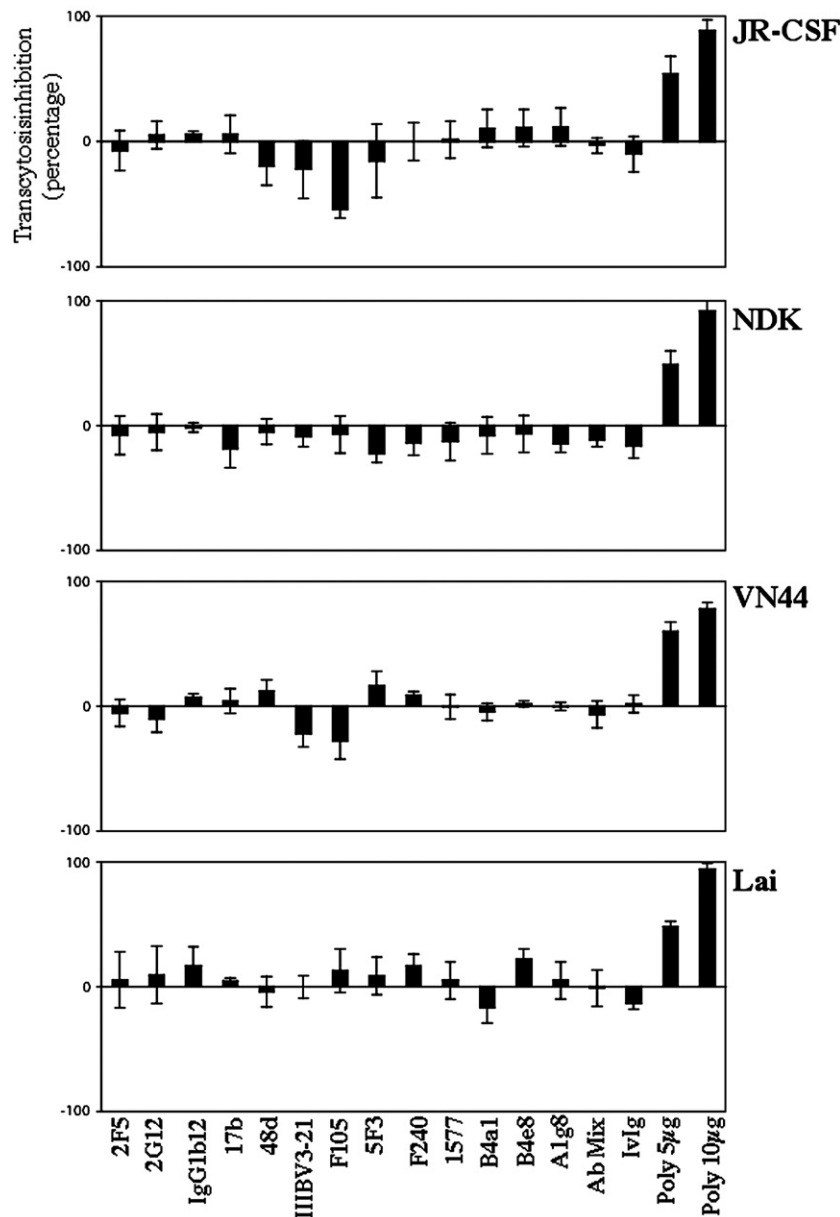


Fig. 3. Inhibition of transcytosis of cell-free HIV-1 through a tight HEC-1 epithelial barrier by 13 monoclonal antibodies and polyclonal antibodies to gp160. Free virus (5 ng of HIV-1_{JR-CSF}, HIV-1_{NDK}, HIV-1_{VN44} and HIV-1_{Lai}) was pre-incubated for 1 h with the indicated antibody (50 µg/ml) and added to the apical medium overlying the monolayer of epithelial cells. Transcytosis was measured by quantitating p24 antigen in the basal medium after 4 h incubation. The results are expressed as means $\pm$ standard deviation of the percentages of transcytosis inhibition obtained in four separate experiments. IvIg: irrelevant IgG; Abmix: Mix of the 13 monoclonal antibodies; Poly: Polyclonal anti-gp160 antibodies.

in a cell-free form (65% versus 88% for HIV-1_{JR-CSF}, 53% versus 92% for HIV-1_{NDK}, 59% versus 78% for HIV-1_{VN44} and 88% versus 94% for HIV-1_{Lai}). Transcytosis of cell-free HIV-1_{JR-CSF} was inhibited by purified polyclonal antibodies to gp160 in a dose-dependent manner (data not shown).

Inhibition of HIV-1 transcytosis by S-IgA and IgG purified from breast milk

We further tested the ability of S-IgA and IgG purified from breast milk from HIV-1-infected mothers demonstrating high titers of anti-gp160 antibodies, to inhibit transcytosis of cell-

associated virus. The addition of purified S-IgA and IgG to HIV-1_{Lai}-infected cells (1.0 µg/2 $\times$ 10⁶ cells) on the apical pole of the epithelial monolayer resulted in significant inhibition of transcytosis. The inhibitory activity of S-IgA was similar to that of IgG on a weight basis, although S-IgA exhibited a lower anti-gp160-specific activity than that of IgG (Fig. 5).

Discussion

None of the 13 HIV-specific monoclonal antibodies tested in the present study, including the three broadly neutralizing antibodies 2F5, 2G12 and IgG1b12, exhibited inhibitory

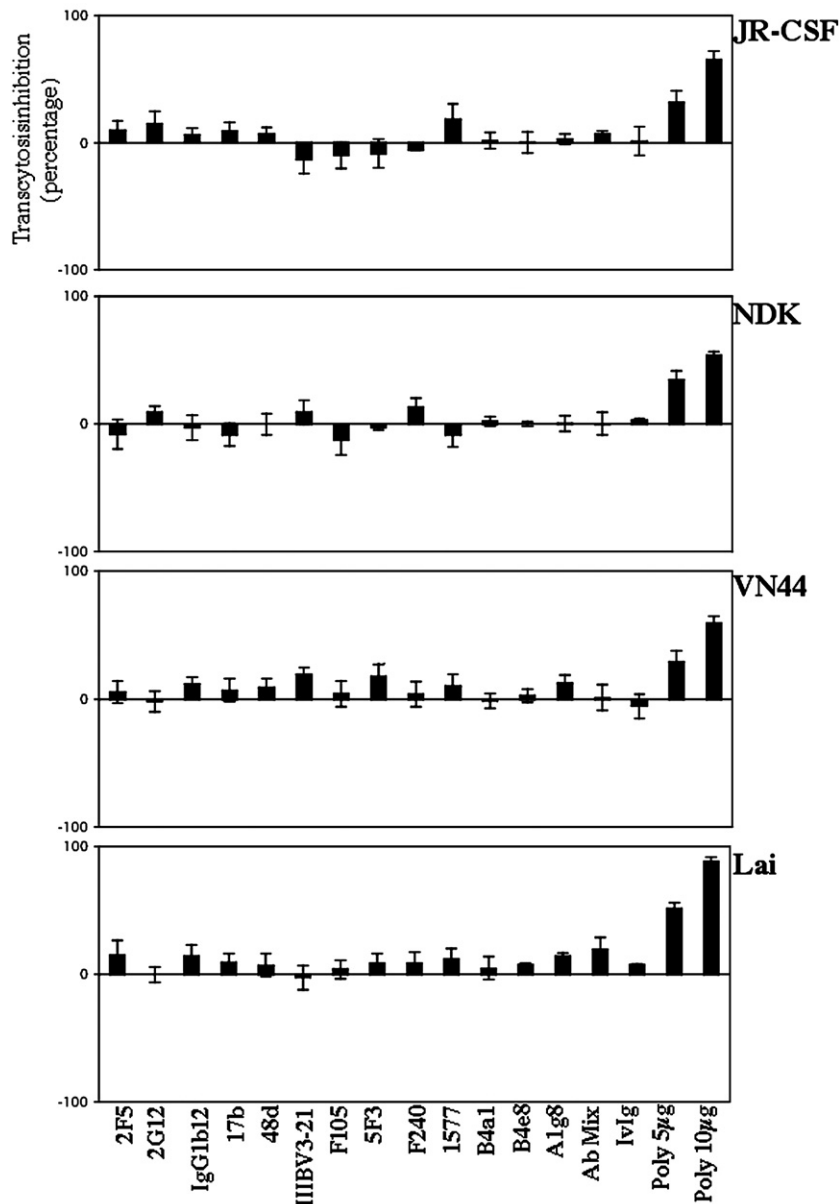


Fig. 4. Inhibition of transcytosis of cell-associated HIV-1 through a tight HEC-1 epithelial cells monolayer by 13 monoclonal antibodies and polyclonal antibodies to gp160. Infected PBL (2×10^6 cells infected by HIV-1_{JR-CSF}, HIV-1_{NDK}, HIV-1_{VN44} or HIV-1_{Lai}) were deposited on the apical surface of monolayer of HEC-1 cells with HIV-specific antibody (50 µg/ml). Transcytosis was assessed as depicted in the legend of Fig. 3. IvIg: irrelevant IgG; Abmix: Mix of the 13 monoclonal antibodies. Poly: Polyclonal anti-gp160 antibodies.

activity towards transcytosis of cell-free and cell-associated R5- as X4-tropic HIV-1 through a tight and polarized monolayer of HEC-1 epithelial cells. Pooling the antibodies did not result in any inhibitory activity either. All 9 monoclonal antibodies known to be neutralizing prevented HIV replication in PBL. As previously shown (Hocini et al., 2001), purified serum polyclonal IgG antibodies to gp160 lead to a potent inhibition of transcytosis of cell-free and cell-associated R5- as X4-tropic HIV. Taken together, these results suggest that the major envelope epitopes involved in neutralization are not involved in mediating HIV transcytosis. The data further indicate that the molecular basis of the transcytosis process of HIV-1 through epithelial cells differs from that of infection of PBMCs with HIV-1.

Transcytosis of cell-free and cell-associated HIV-1 through an epithelial cell monolayer is an active and specific process implying viral envelope glycoproteins (Bomsel, 1997; Hocini et al., 2001). Thus, we first observed that HIV-1 mutant lacking envelope glycoproteins (Δenv) was not capable of transcytosis through HEC-1 cells. We also confirmed previous reports showing that the efficacy of transcytosis is independent of HIV tropism (Hocini et al., 2001). For example, HIV-1_{Ba-L} (R5-tropic) and HIV-1_{89.6} (R5- and X4-tropic) were not able to transcytose through epithelial cells despite their ability to infect CD4-positive lymphocytes and macrophages. Taken together, these findings suggest that transcytosis could depend on amino-acid sequences of gp160, likely not implicated in viral tropism. Hence, the amino-acid sequences in variable domains of gp160

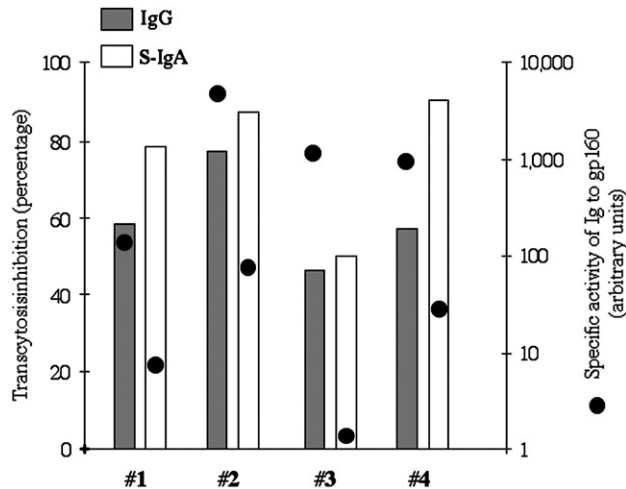


Fig. 5. Transcytosis inhibition provided by secretory-IgA (S-IgA; 1 μ g, colored bars) and IgG (1 μ g, white bars) purified from breast milk collected from 4 HIV-1-infected African mothers (women #1; #2; #3 and #4) (left scale). Specific activities of purified breast milk immunoglobulins (black circles, right scale) were calculated as the ratio of anti-gp160 activity (determined as optical density of anti-gp160 by ELISA) out of the concentration of total immunoglobulin of the same isotype (concentration of IgG or IgA in μ g/ml), and are expressed in arbitrary units.

may determine both the expression of specific gp160 epitopes and differences in affinity between HIV-1 and its cellular receptors. We further confirmed the role of envelope glycoproteins in HIV transcytosis by showing that purified polyclonal antibodies to gp160 demonstrated major inhibitory properties of transcytosis of HIV through a monolayer of HEC-1 cells. Since glycosylation of gp160 was also shown to be critical for an efficient interaction of HIV-1 with target cells, it could be that polyclonal antibodies to gp160, in contrast to monoclonal antibodies, recognize glycosylated epitopes implicated in attachment of HIV-1 to the epithelial cell (Bolmstedt et al., 1996). This latter hypothesis is in part supported by previous observation demonstrating that antibodies to GalCer, an alternative receptor recognizing carbohydrate residues of surface viral glycoproteins (Bhat et al., 1991), are able to partially inhibit HIV transcytosis (Bomsel, 1997) (in our hands, for example, anti-GalCer antibodies were able to inhibit by 25% the transcytosis of free HIV-1_{JR-CSF₂}, data not shown). Finally, the inhibition of transcytosis of cell-associated virus was always less potent than that observed with the corresponding free virus, suggesting that close contact between HIV-1-infected PBL and the apical membrane of epithelial cells probably restricts access of HIV-specific antibodies to their viral epitopes, thus affecting their blocking capability.

The blocking capability on transcytosis of a panel of monoclonal antibodies directed against gp160 was investigated. We selected antibodies recognizing major epitopes implicated in the interaction between the gp160 and the CD4 and the co-receptors CCR5 and CXCR4 (CD4 binding site, CD4i, V3, V4 and gp41). Some of these antibodies (IgG1b12, 2F5 and 2G12) broadly neutralize primary isolates of HIV-1 on PBMC in vitro. None of the antibodies tested was able to block transcytosis of either cell-free or cell-associated virus, even when the antibodies were

pooled. The 2F5 monoclonal antibody was not able to inhibit transcytosis of cell-free and cell-associated R5- as X4-tropic HIV, in accordance with the previous observations reported by Wolbank et al. (2003).

Secretory-IgA purified from colostrum of HIV-1-infected women exhibited up to 250-times lower anti-gp160 activity than did corresponding IgG. In spite of this lower anti-gp160 activity, S-IgA exhibited similar capability to inhibit transcytosis of HIV-1 through a monolayer of epithelial cells. This result is in favor of an important implication of molecular forms or/and valence of anti-gp160 antibodies in inhibition of virus transcytosis. Binding of IgG, dimeric IgA and pentameric IgM polyclonal antibodies on HIV-1 leads to the coating of the viral particle and probably to agglutination of virions at the surface of epithelial cells. This hypothesis is consistent with the results of Wolbank showing that polymeric 2F5 and 2G12, but not the corresponding monomeric antibodies forms, interfere with HIV-1 transcytosis across a mucosal epithelial layer (Wolbank et al., 2003). Alfsen and colleagues have also shown that S-IgA specific to gp41 blocked cell-associated HIV-1 transcytosis (Alfsen et al., 2001). Surprisingly, Wolbank and colleagues reported that high avidity 2F5 IgM, that is the most efficient isotype for agglutination, was less efficient than the corresponding dimeric IgA antibody to inhibit transcytosis. It is likely that limited inhibition induced by 2F5 IgM could be due to the inability of this pentameric immunoglobulin to bind an epitope located at the basis of gp41 ectodomain. In contrast to the limited inhibitory effect of the 2F5 IgM, the 2G12 IgM recognizing a conserved discontinuous motif on the outer domain of gp120 strongly inhibits HIV-1 transcytosis (Wolbank et al., 2003). Taken together, these findings and our present results suggest that surface agglutination of viral particles may participate to the blocking effect observed with both polyclonal and polymeric anti-gp160 immunoglobulins.

The present study demonstrates that anti-envelope antibody-mediated inhibition of HIV-1 transcytosis through polarized epithelial cells likely involves distinct molecular mechanisms from those involved in neutralization. The use of the HIV transcytosis inhibition assay by HIV-specific antibodies to assess the mucosal antibody response to the virus, in highly exposed persistently seronegative individuals and in individuals immunized in vaccine trials, warrant further investigations regarding mechanisms of the inhibition and correlates of protection.

Materials and methods

Reagents and antibodies

The following HIV-specific monoclonal antibodies were obtained through the AIDS Reagent Program, Division of AIDS, NIAID, NIH: anti-HIV-1 gp41 monoclonal antibodies 2F5 (Buchacher et al., 1994; Purtscher et al., 1996, 1994) and 5F3 (Buchacher et al., 1994), anti-HIV-1 gp120 monoclonal antibody 2G12 (Buchacher et al., 1994; Trkola et al., 1996) from Dr Hermann Katinger, anti-HIV-1 gp120 monoclonal antibodies F425 A1g8, F425 B4a1, F425 B4e8 (Cavacini et al., 2003) and F105 (Cavacini et al., 1993; Posner et al., 1993, 1987,

1991), anti-HIV-1 gp41 monoclonal antibody F240 (Cavacini et al., 1998) from Drs Marshall Posner and Lisa Cavacini, anti-HIV-1 gp120 monoclonal antibodies 17b (Kwong et al., 1998; Sullivan et al., 1998; Wyatt et al., 1998) and 48d (Moore and Ho, 1993; Thali et al., 1993) from Dr James E. Robinson, anti-HIV-1 V3 monoclonal antibody IIIB-V3-21 (Laman et al., 1992) from Dr Jon Laman, anti-HIV-1 gp41 monoclonal antibody 1577 (D'Souza et al., 1997; Evans et al., 1989) from Dr Morag Ferguson, anti-HIV-1 gp120 monoclonal antibody IgG1b12 (Barbas et al., 1992; Burton et al., 1991, 1994; Roben et al., 1994) from Drs Dennis Burton and Carlos Barbas. Human polyclonal anti-gp160 antibodies were purified by immunoaffinity from pooled sera of HIV-1 seropositive individuals. Anti-human γ chain was purchased from SIGMA (Sigma Chemical Co., St. Louis, USA). Sepharose beads to which gp160 (MN/Lai) had been covalently bound were incubated for 1 h at 37 °C with undiluted sera. The beads were washed and the antibodies were eluted with 0.2 M glycine-HCl, pH 2.5. The pH of the eluted fractions was immediately adjusted to 7.2 and the reactivity of antibodies to gp160 tested by ELISA, as previously described (Belec et al., 2001).

Secretory-IgA and IgG purification

Colostrum samples were collected at the Complexe Pédiatrique of Bangui, Central African Republic, from four HIV-1-infected mothers. The women were clinically asymptomatic (stage A of the WHO classification) and gave an oral consent for participation in the study. Breast milk samples were centrifuged at 10,000 rpm for 5 min at 4 °C to separate lipids and cells from the acellular fraction. IgG and S-IgA were then purified by immunoaffinity using sepharose beads to which anti-Fc γ or -Fc α (Perbio Science, Brebieres, France) had been covalently bound. Concentrations of total IgG, S-IgA and IgM were determined by ELISA, as previously described (Hocini et al., 1997). Purity of the preparations and specific anti-HIV activity of purified antibodies against gp160 were assessed by ELISA.

Isolation of human lymphocytes

Peripheral blood mononuclear cells (PBMCs) were isolated from buffy coats of healthy donors by centrifugation on Ficoll gradients (Eurobio, Les Ulis, France). Cells were resuspended in RPMI 1640 containing 10% fetal calf serum (FCS). After 1 h incubation, non-adherent cells were removed and further cultured for 48 h in fresh medium supplemented with PHA and IL-2. Peripheral blood lymphocytes (PBLs) were then washed and cultured in growth medium containing IL-2 for 24 h.

Viruses

HIV-1_{NDK}, HIV-1_{VN44} and HIV-1_{JR-CSF} strains were kindly provided by Pr Françoise Barré-Sinoussi (Institut Pasteur, Paris, France). HIV-1_{Ba-L}, HIV-1_{Lai}, HIV-1_{89.6}, HIV-1_{Bru Δ env} and HIV-1_{NL43} were obtained from Dr Caroline Quillent-Grégoire (Hôpital de La Pitié-Salpêtrière, Paris). All viral stocks were

grown in PHA-stimulated PBMCs and quantitated by p24 antigen measurement. Viral strains were titrated using HeLa-CD4-LTR- β -gal cells (AIDS Reagent Program, Division of AIDS, NIAID, NIH, Dr Michael Emerman) and the TCID₅₀ were determined as described (Kimpton and Emerman, 1992).

Neutralization assay

Neutralization of HIV-1 isolates by monoclonal antibodies to gp160 was assessed in PBLs by using an assay measuring the reduction in p24 antigen synthesis by infected cells, as described (Moog et al., 1997). Briefly, virus (0.2 to 1 ng of p24 antigen corresponding to 100 to 1000 TCID₅₀) was incubated with monoclonal antibodies in triplicate in 96-well U-bottom culture plates for 1 h at 37 °C. PHA-stimulated PBL (3×10^5 cells) were added and incubation continued overnight. The cells were then washed three times in RPMI and resuspended in 200 μ l of fresh medium supplemented with IL-2 (R&D Systems). Culture supernatants (50 μ l) were collected at days 3, 6 and 9 and replaced with an equal volume of fresh growth medium. Concentrations of p24 antigen in Triton X-100 lysates were measured using a commercial antigen capture ELISA (Perkin Elmer Life Sciences Inc, Courtaboeuf, France). Neutralization of PBL infection was assessed by measuring the reduction in p24 antigen concentration in culture supernatants during the early phase of virus production (*i.e.* days 3 to 6, depending on the viral strain). Control wells contained virus that had been pre-incubated with RPMI alone (no antibody). In some experiments, additional controls included virus pre-incubated with irrelevant IgG antibody.

Transcytosis assay

Inhibition of transcytosis was measured by means of an *in vitro* system using a polarized monolayer of HEC-1 endometrial epithelial cells (ATCC, Rockville, Maryland, USA) grown in RPMI1640 10% FCS on a 0.4 μ m pore polycarbonate permeable support (Costar, Cambridge, MA), as previously described (Becquart et al., 2000; Belec et al., 2001; Hocini et al., 2001). After 7 to 9 days of culture, the tightness of the monolayer was monitored by measuring resistance (>300 Ohms/cm²) at the apical and basolateral poles of the cells (Ohmeter, Millicell, Millipore). Free virions (5 ng of p24 antigen) were pre-incubated for 1 h in 100 μ l with different concentrations (up to 50 μ g/ml) of each monoclonal antibody. For cell-associated virus transcytosis, HIV-1-infected PBL (2×10^6 cells) that had been washed three-times were added to the apical chamber of the system together with the monoclonal antibody to be tested at the desired concentration (up to 50 μ g/ml). Transcytosis was assessed after 4 h, 24 h and 48 h by measuring p24 antigen concentration in the basal chamber. Inhibition of transcytosis was expressed as percentage of p24 antigen recovered in the basal chamber in the presence of antibodies, compared with the amount of p24 antigen recovered in the absence of antibody. A percentage of inhibition superior to 25% (mean of the values obtained after pre-incubation with irrelevant IgG plus 3 standard deviations) was considered as significant.

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