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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

Hicham Bouhlal,1* Nicolas Chomont, † Mary Réquena,* Nadine Nasreddine,* Héla Saidi,* Jérôme Legoff,* Michel D. Kazatchkine, ‡ Laurent Bélec,* and Hakim Hocini*

In the present study, we demonstrated that opsonization of primary HIV-1 with human complement enhances infection of immature monocyte-derived dendritic cells (iDC) and transmission in trans of HIV to autologous CD4+ T lymphocytes. Infection of iDC by opsonized primary R5- and X4-tropic HIV was increased 3- to 5-fold as compared with infection by the corresponding unopsonized HIV. Enhancement of infection was dependent on CR3 as demonstrated by inhibition induced by blocking Abs. The interaction of HIV with CCR5 and CXCR4 on iDC was affected by opsonization. Indeed, stromal-derived factor-1 was more efficient in inhibiting infection of iDC with opsonized R5-tropic HIV-1 ∆3 (45%) than with heat-inactivated complement-opsonized virus and similarly RANTES inhibited more efficiently infection of iDC with opsonized X4-tropic HIV-1 ∆NDK (42%) than with heat-inactivated complement-opsonized virus. We also showed that attachment of complement-opsonized virus to DC-specific ICAM-grabbing nonintegrin (DC-SIGN) molecule on iDC and HeLa DC-SIGN+ CR3− cells was 46% and 50% higher compared with heat-inactivated complement-opsonized virus, respectively. Hence, Abs to DC-SIGN suppressed up to 80% and 60% the binding of opsonized virus to HeLa cells and iDC, respectively. Furthermore, Abs to DC-SIGN inhibited up to 70% of the infection of iDC and up to 65% of infection in trans of autologous lymphocytes with opsonized viruses. These results further demonstrated the role of DC-SIGN in complement-opsonized virus uptake and infection. Thus, the virus uses complement to its advantage to facilitate early steps leading to infection following mucosal transmission of HIV. The Journal of Immunology, 2007, 178: 1086–1095.

Transmission of HIV-1 occurs following the passage of virus through mucosal epithelial cells, i.e., cervical/vaginal cells upon sexual transmission (1) or intestinal cells upon postnatal transmission by breast milk (2). Semen, cervical/vaginal secretions, and breast milk contain complement proteins that may allow viral particles to be opsonized (3–5). Opsonization of HIV-1 with complement results in enhancement of viral infection of T and B cell lines (6, 7), primary PBMC (8), and primary monocytes/macrophages (9). We have demonstrated that HIV particles activate complement in semen resulting in an ability to infect human CD4-negative epithelial cells in a complement-dependent fashion via CD11b/CD18 (CR3) (10). Recently, others have reported a crucial role of CR3 in the productive infection of dendritic cells (DC) by C3-opsonized HIV (11). However, the precise mechanism through which complement opsonized HIV leads to an increase in both HIV binding to DC and virus production remains unclear.

Immature DCs (iDCs) are a key target for HIV following transmucosal transmission of the virus (12, 13). It has been postulated that certain iDCs present in the peripheral mucosa are the first immune-competent cells to encounter virus particles. Subsequent to virus capture by iDC, infectious HIV particles are transported to the draining lymph nodes. DC differentiated from blood monocytes or CD34+ cells express CD4 and HIV-1 coreceptors CCR5 and CXCR4 (14–16). Although productive infection of certain subset of iDC has been controversial, iDC derived from blood monocytes and skin Langherans cells have been shown to be susceptible to R5- and X4-tropic HIV in vitro (17–20). The attached HIV on DC membrane either infects target cells after interaction with CD4+ coreceptors and/or efficiently transmitted to CD4+ lymphocytes. DCs strongly express CR3 (98%) and other molecules that bind HIV directly through the gp120/gp41 envelope, including DC-specific ICAM-grabbing nonintegrin (DC-SIGN) (21), which is a C-type lectin that binds to high-mannose oligosaccharides on gp160. DC-SIGN is involved in both the attachment of HIV to DC and the transfer of HIV-1 to CD4+ T cells (22).

In the present study we assessed the role of complement receptor CR3 and that of DC-SIGN in the capture of complement-opsonized R5- and X4-tropic HIV by iDC, infection of the cells and transmission of HIV from iDC to autologous CD4+ T cells. We demonstrate that opsonization of virus with complement enhances

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1 Université René Descartes Paris V and Institut National de la Santé et de la Recherche Médicale, Unité 743, and Equipe d’Immunité et Biothérapie Mucqueuse, Institut des Cordeliers, Paris, France; 2 Laboratoire d’Immunologie, Hôpital St. Luc, Centre de Recherches Pavillon Edouard-Asselin, Montréal, Québec, Canada; 3 Instituto Nacional de la Sanit e et la Recherche Médicale, Unité 681, Paris, France

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1 This work was supported by Agence National de la Recherches sur le SIDA, Paris, France.
2 Address correspondence and reprint requests to Dr. Hicham Bouhlal, Institut National de la Santé et de la Recherche Médicale, Unité 743, Equipe d’Immunité et Biothérapie Mucqueuse, Institut des Cordeliers, Escalier E. 2ème Etage, 15 rue de l’École de Médecine, 75270 Paris Cedex 06, France. E-mail address: hicham.bouhlal@u430.bhdc.jussieu.fr
3 Abbreviations used in this paper: DC, dendritic cell; iDC, immature DC; DC-SIGN, DC-specific ICAM-grabbing nonintegrin; AC-OV, activated complement-opsonized virus; HIC-OV, heat-inactivated complement-opsonized virus; SDF, stromal-derived factor; MFI, mean fluorescence intensity.

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the transmission of both R5- and X4-tropic HIV to T cells by facilitating the interaction of the virus with CR3 and DC-SIGN on iDC.

Materials and Methods

Abs and reagents

PE-conjugated anti-CCR5 (2D7), anti-CXCR4 (12G5), and PE-conjugated anti-CD4 (Leu3-a) were obtained from BD Pharmingen; PE-conjugated anti-CD11b, FITC-conjugated anti-CD11c (CR4), anti-CD35 (CR1) and anti-CD21 (CR2), endotoxin-free preparations of blocking CD11b (MO-1), blocking CD18 (7E4) and the corresponding isotypes, FITC-conjugated anti-CD83, PE-conjugated anti-CD1a, and goat anti-mouse Ab were purchased from Immunotech. Unconjugated mouse anti-DC-SIGN blocking mAbs (DC-4 and DC-6) were obtained from the AIDS Reagent Program, National Institute of Allergy and Infectious Diseases (NIAID, National Institutes of Health, Bethesda, MD). FITC-conjugated anti-DC-SIGN, anti-MHC class I HLA-DR anti-CD3, and PE-conjugated anti-CD14 are obtained from BD Pharmingen. The FITC-conjugated rabbit anti-gp120 and corresponding matched isotype were purchased from Intracell. Cultures were grown in RPMI 1640 supplemented with 100 U/ml penicillin, 100 mg/ml streptomycin (Invitrogen Life Technologies), and 10% heat-inactivated FCS (Dutscher). Recombinant human IL-2, IL-4, GM-CSF, RANTES, and stromal-derived factor (SDF)-1 were obtained from R&D Systems. Azidothymidine, PHA, and polyclonal anti-C3 complement fragment were obtained from Sigma-Aldrich. Normal human serum used as a complement source consisted of pooled sera of healthy individuals.

Cells primary cultures and HIV-1 sources

DCs were differentiated from PBMC. After step-density gradient centrifugation, PBMC (10^7 cells/ml) were cultured in RPMI 1640/10% of normal human serum for 1 h at 37°C. After washing, adherent cells were maintained in RPMI 1640/10% FCS, 1% antibiotics supplemented with IL-4/GM-CSF (both at 10 ng/ml) to obtain iDC. Medium was changed every 48 h and new cytokines IL-4/GM-CSF added to the medium. Contamination of iDCs with CD3^-/H11001^-T lymphocytes was 0.1% as checked by FACS.

Autologous lymphocytes were obtained by stimulation of the nonadherent cell fraction of PBMC with PHA/IL-2 (2.5 g/ml; 10 IU) for 72 h. Primary X4-tropic HIV-1NDK was grown in PHA/IL-2-activated PBL. R5-tropic HIV-1Bal was amplified in macrophages cultures. Virus particles were quantified by measuring p24 HIV protein (HIV-p24 ELISA; DuPont). Tropic of viruses was determined using U87 cells positive for CD4 and CXCR4 or CCR5, obtained from the AIDS Reagent Program, NIAID, National Institutes of Health. The TCID_{50} of each strain was measured and virus stored at ~80°C.

Cell staining

Cells (0.5 × 10^6 cells per assay) were collected in cold PBS/0.01% sodium azide/0.5% BSA, washed and incubated with conjugated monoclonal or isotype-matched Abs for 30 min at 4°C. Following a washing step in PBS/0.01% sodium azide, cells were fixed with 1% paraformaldehyde, and 5000 events were analyzed using a FACSCalibur and the CellQuest software (BD Biosciences).

Opsonization of HIV-1 and infection of iDC

Both R5 and X4 HIV-1 particles (1 ng/ml p24), corresponding to 5 × 10^2 TCID_{50}, were opsonized by activated complement-opsonized virus (AC-OV) by adding a similar volume of normal human serum for 1 h at 37°C, containing veronal buffer (0.6 mM CaCl_2 and 0.9 mM MgCl_2 (VBS2)). As negative control, HIV-1 particles were incubated with heat-inactivated complement-opsonized virus (HIC-OV) serum and in some experiments with medium (unopsonized virus).

Treated HIV-1_{NDK} or HIV-1_{Bal} (1 ng/ml HIV p24) were then incubated with iDC (5 × 10^5 cells) for 3 h at 37°C. In some experiments, cells were incubated, with endotoxin-free preparations of blocking CR3 Abs (7E4 for CD18; MO-1 for CD11b used separately or in combination at 10 μg/ml), receptors expressed on iDC at day 6. B. Dot plot of CD11b, CD11c, CD35, and CD21 expression. The mean percentage of positive cells (%) calculated from ten separate experiments is indicated in corresponding quadrant. FL-1 and FL-2 correspond to the isotype Ab conjugated to the corresponding fluorochrome (FITC or PE).
with human recombinant chemokines SDF-1 and RANTES (2.5 μg/ml), with Abs anti-DC-SIGN (10 μg/ml), for 30 min at room temperature prior to infection. The cells were intensively washed with RPMI 1640 to eliminate unbound virus, serum and blocking Abs excess, and cultured in fresh RPMI 1640 containing FCS for 6 days in absence of additional cytokines or Abs. Residual HIV p24 released by iDC in the culture medium has been estimated after inhibition of viral replication by azidothymidine molecule at 5 μM. Culture supernatants were collected every 48 h and viral content monitored by means of HIV-1 p24 ELISA. In coculture assays, iDCs were incubated with virus for 1 h at 37°C, and washed before the addition of autologous IL-2-stimulated lymphocytes at a 1:5 ratio of iDC to lymphocytes. Cocultures were maintained in the presence of IL-2 for 6 days.

Detection of HIV-1 DNA in infected iDC

Amounts of HIV-1 DNA in iDC were quantified at 6 h and 6 days following infection with opsonized or unopsonized HIV used at 1 and 5 ng/ml p24 Ag. DNA was extracted from washed cellular pellets using the Qiagen QIAamp DNA mini kit, according to the manufacturer’s instructions. A semiquantitative PCR of the pol gene was conducted as previously described (23). The PCR was performed with the oligonucleotides P63 (5’-GCC ATT TAA AAA TCT GAA AAC AGG-3’) and P58 (5’/H11032-GAC AAA CTC CCA CTC AGG AAT CCA-3’) for 37 cycles (94°C for 30 s, 53°C for 30 s, and 72°C for 60 s) in a reaction volume of 50 μl, containing Milli-Q water, DNA templates, reaction buffer (1.5 mM MgCl2), 200 μM each dNTP, 250 nM of each primer, and 2.5 U of TaqDNA polymerase) provided by the manufacturer (Promega). To check for the quality of extracted DNA and the lack of PCR inhibitors, the ubiquitous β-globin gene was amplified in parallel experiments by PCR, as described (24). The final PCR products were visualized under UV transillumination by means of ethidium bromide staining after electrophoresis in 2% agarose.

Attachment of HIV to DC-SIGN on HeLa cells and iDC

The HIV-nonpermissive HeLa cells that stably express DC-SIGN molecule (90–95%) were used to evaluate the HIV DC-SIGN-dependent attachment in absence of complement receptors, HIV receptors, and coreceptors. HeLa cells (5 × 10⁶ cells) were incubated with opsonized or unopsonized HIV-1 induces (10 ng/ml HIV p24) for 1 h at 37°C. Cells were washed with RPMI 1640 to remove unbound virus and lysed by adding 200 μl of PBS-Triton X-100 at 0.5%, before quantification of HIV p24 Ag. In some experiments, cells were incubated with a combination of DC-SIGN-blocking mAbs (DC-4/DC-6) at 10 μg/ml and polyclonal anti-C3 complement component (10 μg/ml) before addition of virus. The DC-SIGN-negative HeLa cells were used to evaluate the DC-SIGN-independent HIV binding. In additional experiments, iDC (10⁶ cells) were preincubated with DC-SIGN-blocking mAbs (DC-4/DC-6) or blocking CD11b mAb (MO-1) for 30 min at room temperature before adding HIC-OV and AC-OV HIV-1BaL (10 ng/ml HIV p24) for 1 h at 37°C. Cells were then washed and incubated with FITC-conjugated anti-gp120 and PE-conjugated anti-CD1a mAbs for 1 h at 4°C. Following a washing step in PBS/0.01% sodium azide, cells were fixed with 1% PFA and 5000 events were analyzed using a FACScalibur and the CellQuest software. HIV binding levels were expressed as gp120 stained cells.

Statistical analysis

Quantitative analysis is expressed as the mean and SE. An unpaired Student’s t test was used to determine the statistical significance of the data and a value for p < 0.05 was considered as the level of statistical significance.

Results

Immature DCs are susceptible to infection with both primary R5- and X4-tropic HIV-1

At the time of collection on day 6 of culture, most nonadherent mononuclear cells exhibited an iDC phenotype (CD14+, HLA-DR+, CD1a+) expressing low levels of CD83. The iDC expressed...
CD4 at 70 ± 20%, CCR5 at 20 ± 10%, CXCR4 at 30 ± 15%, and up to 80 ± 10% DC-SIGN molecule (Fig. 1A). The iDC expressed high levels in mean fluorescence intensity (MFI) of both CR3 (98%, 700–800 MFI) and CR4 (80%, 250–300 MFI). Only 0.2% and 20% of the cells expressed CR2 and CR1, respectively (Fig. 1B). HIV infection of iDC was observed with both X4-tropic (HIV-1NDK) and R5-tropic (HIV-1BaL) strains (Fig. 2A). A plateau of p24 Ag production in cultures was reached at day 6 after infection. At the peak of infection (day 6) with HIV-1BaL and HIV-1NDK, the amounts of HIV p24 Ag released in culture supernatants of 5 × 10^5 cells were of 2500 ± 560 and 500 ± 55 pg/ml, respectively. To evaluate the amount of HIV particles adsorbed and/or internalized in the absence of infection, cells were cultured in the presence of 5 μM azidothymidine, an inhibitor of the viral retro transcription step. The results showed that <2% of input virus (1 ng/ml p24) corresponding to 20 pg/ml HIV p24 was detected in iDC-infected cultures. Infection of iDC, by HIV was further confirmed by nested PCR on HIV pol DNA at 6 h and at day 6 postinfection (Fig. 2B).

**CR3-dependent enhancement of iDC infection by opsonized HIV-1**

Complement opsonization of HIV-1 particles resulted in a 3- to 5-fold increase in HIV p24 production in culture supernatants at

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**FIGURE 4.** Inhibition by CR3 blocking Abs of complement-dependent infection of iDC and transmission in trans to autologous CD4^+^ T lymphocytes. Cells were preincubated with a combination of CD11b and CD18 blocking Abs (mAb anti-CR3) or matched isotypes all used at 10 μg/ml, before addition of AC-OV and HIC-OV. Immature DC infected with HIV_BaL. (A) and HIV_NDK (B) iDC infected with HIV_BaL and HIV_NDK and cocultured with IL-2-activated autologous lymphocytes at a 1:5 ratio (iDC to T cell). The viral production was quantified by p24 production in cell supernatants at day 6. Results are presented as the mean ± SD of p24 concentrations from five separate experiments. Statistical significance was calculated for p24 production at day 6 in the presence and absence of anti-CR3 Abs, using the unpaired Student t test and considered significant at a value of p < 0.05.
day 6 postinfection as compared with production observed in the absence of opsonization of HIV (Fig. 3A). No enhancement of infection was observed when viruses had been incubated with iDC for 30 min at room temperature before infection by AC-OV or HIC-OV of HIV-1\textsubscript{Bal} and HIV-1\textsubscript{NDK} (1 ng/ml p24). Viral production was quantified at day 6 postinfection by HIV p24 ELISA. Results are presented as the mean ± SD of p24 concentrations calculated in picograms per milliliter from five separate experiments. Statistical significance was calculated using the unpaired Student t test and considered significant for values of $p < 0.05$. *, $p < 0.03$; **, $p < 0.02$.

FIGURE 5. Inhibitory effect of SDF-1 and RANTES chemokines on infection of iDC with complement-opsonized virus. SDF-1 (A) and RANTES (B) at 2.5 μg were preincubated with iDC for 30 min at room temperature before infection by AC-OV or HIC-OV of HIV-1\textsubscript{Bal} and HIV-1\textsubscript{NDK} (1 ng/ml p24). Viral production was quantified at day 6 postinfection by HIV p24 ELISA. Results are presented as the mean ± SD of p24 concentrations calculated in picograms per milliliter from five separate experiments. Statistical significance was calculated using the unpaired Student t test and considered significant for values of $p < 0.05$. *, $p < 0.03$; **, $p < 0.02$.
and HIV-1\textsubscript{NDK} (6500 vs 1500 pg/ml for HIV-1\textsubscript{Bal} and 1900 vs 700 pg/ml HIV p24 for HIV-1\textsubscript{NDK} in the absence and presence of blocking Abs, respectively) (Fig. 4A). The role of CD11b and CD18, \textbeta and \alpha chains forming CR3, in the amplification of iDC infection with complement-opsonized virus has also been investigated. We found that the anti-CD11b (MO-1) used alone achieves 60–85% of inhibition, whereas anti-CD18 (7E4) inhibits <20% the infection with AC-OV (data not shown). In following inhibition experiments, only a combination of both Abs (MO-1 plus 7E4; 10 \mu g/ml each) was used. When autologous IL-2-activated lymphocytes were added (in 1:5 ratio) to iDC that had been incubated with anti-CR3 Abs before being infected, we observed an inhibition of 65% and 60% of the transmission in trans of AC-OV HIV-1\textsubscript{Bal} and HIV-1\textsubscript{NDK} to CD4 cells, respectively. No significant inhibition was observed when CR3-blocking Abs were used with HIC-OV HIV-1\textsubscript{Bal} and HIV-1\textsubscript{NDK} (Fig. 4B). Isotype-matched Ab controls had no effect on infection and transmission in trans of HIV.

Opsonization of HIV-1 with complement modulates the usage by HIV of CCR5 and CXCR4

The effect of complement opsonization on the use of the coreceptors CCR5 and CXCR4 by HIV was investigated by incubating iDC with RANTES and SDF-1 before the addition of either AC-OV or HIC-OV. Cells were cultured in absence of additional cytokines, and p24 concentration was determined by ELISA at day 6 after infection. Our results showed that SDF-1 inhibited more efficiently infection of iDC with AC-OV R5-tropic HIV-1\textsubscript{Bal} (53% of inhibition) than with the HIC-OV (<1%) (p < 0.03). Similarly, RANTES inhibited at 56% and <5% infection of iDC with AC-OV and HIC-OV X4-tropic HIV-1\textsubscript{NDK}, respectively (p < 0.02). In contrast, SDF-1 was less potent in inhibition of iDC infection with AC-OV X4-tropic strain HIV-1\textsubscript{NDK} (51%), than HIC-OV (90%) (p < 0.02%). RANTES inhibited equally infection of iDC with AC-OV and HIC-OV R5-tropic strain HIV-1\textsubscript{Bal} (80% of inhibition vs 78% for HIC-OV) (Fig. 5B). Preincubation of iDC with monoclonal anti-CD4 Ab inhibited infection with
AC-OV and HIC-OV X4-tropic and R5-tropic viruses at a similar degree (80%) (data not shown).

Complement opsonization enhances the transmission of HIV from iDC to CD4+ T lymphocytes through increased attachment to DC-SIGN

Complement opsonization of HIV resulted in a 2- to 3-fold enhancement of infection in trans of T lymphocytes when these cells were added to iDCs that had been infected with AC-OV compared with HIC-OV. We examined the role of DC-SIGN in infection of iDC and in their ability to transmit virus to autologous T cells in the presence of AC-OV and HIC-OV. Preincubating with anti-DC-SIGN Abs (DC-4 and DC-6 at 10 µg/ml each) resulted in a greater inhibition of infection of iDC with AC-OV R5-tropic HIV-1\textsubscript{BaL} (55%) than in the case of the corresponding HIC-OV (10%). A similar result was observed when infection was conducted with AC-OV X4-tropic HIV-1\textsubscript{NDK} (65%) as compared with the corresponding HIC-opsonized virus (15%) ($p < 0.02$) (Fig. 6A). We further found that anti-DC-SIGN Abs induced a significantly higher inhibition of transmission of AC-OV R5-tropic HIV-1\textsubscript{BaL} (60%) and X4-tropic HIV-1\textsubscript{NDK} (70%) to autologous T cells than of HIC-OV HIV-1\textsubscript{NDK} (58%) and HIV-1\textsubscript{BaL} (46%) (Fig. 6B). To
highlight the mechanism by which complement-opsonized HIV infects more efficiently iDC, we have investigated the role of DC-SIGN using a transfected epithelial HeLa cell line that stably express DC-SIGN molecule but does not express CR1, CR2, CR3, and CR4 complement receptors. As shown in the Fig. 7A, HIV complement opsonization enhances up to 50% of the attachment of virus to DC-SIGN+ HeLa cells as compared with HIC-OV. The attachment of complement-opsonized HIV occurred through DC-SIGN as demonstrated by the 80% of inhibition induced by Abs directed to DC-SIGN. Furthermore, polyclonal Abs directed against C3 component fragment induced also an inhibition of 60% of attachment of opsonized virus (Fig. 7A). As control the binding of HIC-OV and AC-OV to DC-SIGN-negative HeLa cells represented <5% of specific attachment observed with HeLa DC-SIGN-positive cells. The role of DC-SIGN molecule has been also investigated using iDC incubated with HIC-OV and AC-OV. Our results showed that the positive cells stained by FITC-conjugated anti-gp120 Abs increased from 20% to 66% (corresponding to 70% of HIV binding enhancement) when HIC-OV and AC-OV were used, respectively (Fig. 7B). The anti-DC-SIGN and anti-CR3 blocking Abs decreased the binding of AC-OV from 66% to 28% (58% of inhibition) and to 32% (50% of inhibition), respectively. In contrast, the binding of HIC-OV (20%) was not significantly changed in the presence of Abs anti-DC-SIGN and anti-CR3. As control, matched isotype Abs achieved <5% of inhibition of HIV attachment. Down modulation of expression of iDC marker CD1a in the presence of HIV and complement fragment is probably due to the maturation priming of immature DC.

Discussion

In the present study, we demonstrate that opsonization of HIV with complement enhances both the infection of iDC and the transmission in trans of virus to CD4+ T lymphocytes. Enhancement of infection of iDC observed following preincubation of virus with normal serum was complement-dependent and occurred with both R5- and X4-tropic primary isolates of HIV-1. Thus, productive infection of iDC was seen much earlier and was 5-fold higher with AC-OV than in the presence of corresponding HIC-OV and unopsonized virus. The virus produced in supernatant at day 6 was infectious as demonstrated by productive infection of IL-2-activated lymphocytes. In addition, proviral HIV-1 DNA was detected in iDC within 6 h of incubation with complement-opsonized HIV-1, the amounts of HIV-1 pol DNA measured by semiquantitative nested PCR being significantly higher in cells infected with opsonized HIV, as compared with cells infected with virus that had been preincubated with culture medium. These data extend previous observations on the enhancing effect of complement on infection of monocytes/macrophages (8, 9) and more recently of CD4-negative, complement receptor-positive epithelial cells (10). The role of complement receptor (CR3) was shown by the blocking effect of anti-CR3 mAbs on infection of iDC with opsonized HIV-1SDF and HIV-1NDK. The fact that anti-CD11b and anti-CD18 mAbs inhibited 70–80% and <20%, respectively, infection of iDC by opsonized virus, is in favor of implication of α4 integrin and β2 chains (CR3) in interaction of opsonized virus with iDC membrane. However, the opsonized virus may also interact with the CR4 molecules that are also expressed on iDC (250–300 MFI for CD11c compared with 700–800 MFI for CD11b). Investigating this situation is complicated by the fact that no neutralizing Abs against CR4 receptor are available. In contrast, the anti-CD11a (LFA-1) Abs inhibited <10% of infection with opsonized virus. Several regions in gp120/gp41 including the conserved C2 region, the carboxyl-terminal flank of V3 loop and the transmembrane gp41 domain have been shown to support complement activation through a direct interaction with C1q and/or C3 (25–28). Activation of complement results in the deposition of C3 fragments on the viral surface without efficient formation of the C5b-9 terminal complex and without lysis of the virus because of the presence of complement regulatory proteins on the viral membrane (27–29). Opsonized virus can divert the complement system to its advantage by using the CR3/CR4 receptor to infect iDC and other complement receptor-expressing cells (30). Furthermore, binding of complement-opsonized HIV to CR3 may induce specific signaling cascades in iDCs, which are favorable for HIV replication. We further investigated the role of opsonization of HIV in facilitating the transmission in trans of virus from iDC to autologous CD4+ T cells. We observed that opsonization of HIV enhanced by 2- to 3-fold viral transfer to CD4+ T cells that do not express CR3 molecule. Enhancement of viral transmission was in part dependent on CR3 on iDC because anti-CR3 mAbs inhibited by ~60% transmission in trans of opsonized HIV-1pol and HIV-1NDK. It is known that Abs directed against CR3 (MO-1 plus 7E4) exert a blocking effect on CR3 function and binding of C3 components, in the absence of cross-linking by a secondary Ab. The role of CD11b and CD18, β and α chains forming CR3, in the infection in trans of C4 T cells with complement-opsonized virus has been investigated separately. To avoid any interference of anti-CR3 Abs with receptors expressed on T cells, iDCs that had been preincubated with MO-1 and 7E4 were washed before being added to target CD4 T cells. We found that the anti-CD11b (MO-1) used alone achieves 60% of inhibition, whereas anti-CD18 (7E4) inhibits <10% the infection with AC-OV (data not shown). This effect is very specific because no significant inhibition was observed when CR3-blocking Abs were used with HIC-OV HIV-1pol and HIV-1NDK.

Because mucosal secretions contain active complement components (5, 31) one may speculate that virus released in the mucosal lumen becomes opsonized and acquires an enhanced potential for attachment to and infection of iDC, a key target cell for HIV in the early steps of sexual transmission of the virus (12). Furthermore, complement opsonized virus already attached to iDC, may interact with CR1 complement receptor expressed on CD4+ T lymphocytes inducing an enhancement of infection in trans of autologous CD4+ T lymphocytes (7). Interestingly, complement receptors were also shown to play a role in the capture and docking of virus on the surface of uninfected follicular DCs in germinal centers (32, 33). However, CR2 rather than CR1 and CR3, was shown to be the main binding molecule for opsonized HIV on follicular DCs (34).

We further investigated the role of CD4, CXCR4, CCR5, and DC-SIGN in the infection of iDC with unopsonized and complement-opsonized HIV. We have shown that the iDC express CD4, CCR5, and CXCR4 and are susceptible to the productive infection by both R5- and X4-tropic strains. This result is confirmed by the recent observation demonstrating that X4-tropic strains replicate in iDC (20). We demonstrate for the first time, that complement opsonization of HIV modulates the inhibitory activity of RANTES and SDF-1 on infection of iDC with X4- and R5-tropic viruses. Indeed, SDF-1 was significantly more efficient in inhibiting infection of iDC with the opsonized R5-tropic HIV-1polR (45%) than with the corresponding unopsonized virus (<1%). Similarly, RANTES was more efficient in inhibiting infection of iDC with opsonized X4-tropic HIV-1polX442% than with the unopsonized virus (<5%). Furthermore, HIV complement opsonization reduced significantly the effect of TAK-779, a CCR5 antagonist molecule, on infection of DCs by CCR5-tropic HIV (data not shown).

The modulation of virus tropism by complement opsonization is probably not the result of an alteration of coreceptors expression...
consequently to the binding of complement fragments to DCs. Indeed, we have previously shown that the membrane expression of CD4, CCR5, and CXCR4 was not affected by the presence of complement components (9). Our results, both on epithelial cells and DCs, are in favor of an effect of opsonins on the interactions between gp160 and coreceptors. Our results suggest that covariant interaction between major viral glycoprotein (gp120/gp41) and the C3 fragments (C3b) induces a modification in interaction of HIV and coreceptors used in viral entry into iDC. Three major sites on gp120 are able to activate the complement system: the second constant region of gp120 C2 (233–251), the third constant region of gp120 and the third variable region V3 loop. These regions are able to activate complement in the absence of Abs (26). Interestingly, the variable V3 loop is also responsible for viral tropism determination allowing an interaction with one of two major HIV coreceptors CCR5 and CXCR4. Thus, it is implied that V3 loop and C2-C3 in interaction with iC3b probably modulate the coreceptors recognition on target cell membrane. In contrast, blocking mAb directed to CD4 receptor did not exhibit a differential effect on infection of epithelial HeLa DC-SIGN and R5-tropic HIV-1NDK and R5-tropic HIV-1DL. This result agrees with the observation that the CD4 binding site on gp120 does not implicate complement-activating sites such as C2, V2, C5, and V3 loop (35). Opsonization of HIV by complement probably enhances the binding to CR3 receptor and increases the number of viral particles adsorbed on cells allowing a more efficient fusion and viral entry through CD4 and HIV coreceptors. All these observations indicate that opsonization with complement alters the phenotype of HIV and transform it from a strict R5- and X4-tropic to a dual tropic virus that allows HIV to use both CCR5 and CXCR4 coreceptors. The latter observation is not in favor of a selection process of R5-tropic HIV during the early phases of mucosal penetration of HIV, suggesting that it occurs at later stages of the infection. Interestingly, as for iDC, the inhibitory effect of RANTES was increased in the case of infection of CD4-negative HT-29 epithelial cells with opsonized X4-tropic HIV-1 (10). However, the inhibitory effect of SDF-1 was decreased in the case of both opsonized X4- and R5-tropic viruses, suggesting that opsonized virus may not interact in a similar fashion with CCR5 and CXCR4 on epithelial cells and iDC.

During the early steps of HIV transmission, iDC and CD4+ T lymphocytes synergize in facilitating initial viral replication. Immature DCs capture and transmit virus to CD4 T lymphocytes leading to a greater sensitivity to the infection of these cells (34–36). HIV-free particles attach to iDC through several molecular pathways, including C-type lectin receptors, e.g., the DC-SIGN molecule that is well expressed by DCs of human genital mucosae (36). It is now admitted that DC-SIGN contributes to the capture virus responsible for transmission to T cells shortly after viral uptake. In contrast, virus produced by infected DCs contributes to prolonged HIV transmission to T cells. In our experimental conditions, the viral production of iDC alone was up to 30-fold times lower than virus production in coculture medium of DC and CD4 T cells. Hence, infection of T cells is largely due to virus released by infected T cells and less to virus produced by infected iDC. In this study we showed that opsonized virus attached more efficiently to DC-SIGN than the corresponding unopsonized virus. Blocking mAbs to DC-SIGN (DC-4 plus DC-6) that block necked domain and C terminus region of DC-SIGN, inhibited more efficiently infection of iDC and transmission to autologous CD4 T lymphocytes of opsonized R5-tropic HIV-1DL and X4-tropic HIV-1NDK than unopsonized corresponding virus. To further demonstrate that opsonized HIV binds more efficiently to DC-SIGN, we used a transfected epithelial HeLa cell that stably expresses DC-SIGN but does not express CR1, CR2, CR3, and CR4 complement receptors. We demonstrated that AC-OV attached up to 2-fold greater than HIC-OV and unopsonized virus. The attachment of complement-opsonized HIV to HeLa cells occurred through DC-SIGN as demonstrated by 80% inhibition of attachment observed in the presence of Ab to DC-SIGN. Furthermore, polyclonal Ab directed against C3 fragment of complement inhibited at 58% attachment of opsonized virus to HeLa DC-SIGN cells. The role of DC-SIGN molecule was also confirmed using iDC. Indeed, the binding of complement opsonized HIV to iDC was decreased by 50% in the presence of anti-DC-SIGN Abs. These results are in favor of a direct interaction between complement fragments and DC-SIGN molecule. Hence, the better interaction of DC-SIGN with complement opsonized HIV gp120 results in a higher ability of virus to attach to HeLa DC-SIGN+ cells and to iDC. HIV complement activation may also occur through the lectin pathway where virus binds mannose-binding lectin present in serum. One may suggest that mannose-binding lectin bind to HIV gp120 before interaction with DC-SIGN expressed on DCs. Interestingly, it has been reported that mannose-binding lectin acts as recognition molecules for infectious agents that colonize the cervicovaginal mucosa, suggesting that the lectin pathway of complement activation may play a crucial role in HIV transmission in female genital tract (37).

Taken together, our results indicate that complement opsonization of viral particles affects the interactions of HIV with DC-SIGN, CCR5, and CXCR4. HIV-1 would thus turn complement opsonization to its own replicative advantage, resulting in enhanced infection of iDC and transmission to CD4 T lymphocytes. In summary, we demonstrate that complement-opsonized HIV acquires a dual tropism for CCR5- and CXCR4-expressing cells and the ability to attach to and infect iDC through complement receptors (CR3) and DC-SIGN-dependent pathways. Thus, the virus probably uses active complement in secretions to facilitate early steps leading to infection following mucosal transmission of HIV.

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Disclosures

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References


