IgG Opsonization of HIV Impedes Provirus Formation in and Infection of Dendritic Cells and Subsequent Long-Term Transfer to T Cells[1]

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Already at initial phases of infection, HIV is coated with complement fragments. During the chronic phase, when HIV-specific IgGs appear, the virus circulates immune complexed with IgG and complement. Thus, we studied the interaction of dendritic cells (DCs) and DC-T cell cocultures with complement (C)-opsonized and C-IgG-opsonized HIV. HIV infection of monocyte-derived DCs and circulating BDCA-1-positive DCs was significantly reduced upon the presence of virus-specific but non-neutralizing IgGs. DCs exposed to C-Ig-HIV or IgG-opsonized HIV showed an impaired provirus formation and p24 production and a decreased transmission rate to autologous nonstimulated T cells upon migration along a chemokine gradient. This reduced infectivity was also observed in long-term experiments, when T cells were added delayed to DCs exposed to IgG-coated HIV without migration. Similar kinetics were seen when sera from HIV-1-infected individuals before and after seroconversion were used in infection assays. Both C- and C-IgG-opsonized HIV were captured and targeted to a tetraspanin-rich endosome in immature DCs, but differed with respect to MHC class II colocalization. The reduced infection by IgG-opsonized HIV is possibly due to interactions of virus-bound IgG with FcγRIIb expressed on DCs. Therefore, the intracellular fate and transmission of immune-complexed HIV seems to differ depending on time and opsonization pattern. *The Journal of Immunology,* **2007, 178: 7840–7848.**

Human immunodeficiency virus attaches to immature dendritic cells (iDCs)[3] that are characterized by their high capacity to take up Ag via endocytic and phagocytic receptors. The captured Ag is degraded by dendritic cells (DCs) that migrate to lymphoid organs, where they prime naive T cells (1). During this process, DCs undergo maturation and their Ag-capturing machinery is down-regulated, whereas their T cell stimulatory capacity significantly increases due to up-regulation of costimulatory and MHC molecules.

Several Ag-capturing receptors are expressed on iDCs, e.g., C-type lectins, FcRs, complement receptors (CRs), and TLRs (2–8). As shown recently by Boruchov et al. (9), human monocyte-derived DCs as well as circulating myeloid DCs coexpress stimulatory (CD32a) and inhibitory (CD32b) isoforms of FcγRII (CD32) on their surface. The balance between CD32a and CD32b on DCs determines their activation status. Ligation of CD32a triggers tyrosine phosphorylation of a cytoplasmic ITAM. In contrast, cross-linking CD32b with immune-complexed IgG induces tyrosine phosphorylation of its cytoplasmic ITIM, thus down-modulating activating events (10). Although FcRs induce signals and mediate uptake constitutively after ligand binding, CR3 requires additional stimuli such as various inflammatory mediators or extracellular matrix proteins after binding iC3b-opsonized ligands (11).

At the beginning of infection, HIV-1 is only confronted with components of innate immunity, such as the complement system. Thus, already at initial phases of infection, HIV is coated with complement fragments. Several weeks after infection with HIV-1, adaptive immunity is fully activated, which is reflected by the generation of specific anti-HIV-1 Abs and activated T cells. Bound Abs enhance the activation of complement and increase deposition of complement fragments on virions dramatically; opsonized infectious virus accumulates in HIV-1-positive individuals. In vivo, immune complexes were identified in blood and on lymphoid follicles, which contained infectious viral particles (12–15). In vitro, both Abs and complement fragments have been shown to mediate attachment of HIV to FcR-/CR-expressing cells and transmission of opsonized virus to permissive $CD4^+$ cells (16–24). Highly purified populations of myeloid and plasmacytoid DCs from blood of untreated patients contained HIV-1 provirus (25), whereas no proviral DNA or viral particles were found in peripheral blood DCs from HIV-1-infected individuals treated with highly active antiretroviral therapy (26). In vivo, after seroconversion, when anti-HIV-IgGs appeared, productively infected DCs are rarely detected (27).

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[3] Abbreviations used in this paper: iDC, immature dendritic cell; DC, dendritic cell; CR, complement receptor; VCA, virus capture assay; NHS, normal human serum; o/n, overnight; SN, supernatant.

Recently, our laboratory demonstrated that complement coating of HIV-1 (in the following termed C-HIV) enhanced infection of iDCs compared with nonopsonized virus (HIV) (24). Inhibition of HIV-1 replication in iDCs by purified anti-HIV-1 IgGs was recently described (28, 29).

Because DCs express not only activating and inhibitory FcγRs but also CR3 and CR4, we have systematically analyzed the fate of different forms of opsonized virus (HIV, C-HIV, HIV opsonized with virus-specific IgGs (Ig-HIV), complement-IgG-opsonized HIV (C-Ig-HIV)) in DCs. Our findings revealed significant differences in the capacity of differentially opsonized virus to infect DCs as well as DC-T cell cocultures.

Materials and Methods

Reagents

Purified GM-CSF was purchased from Schering-Plough, recombinant human IL-4 was obtained from PromoCell, and LPS (*Escherichia coli* serotype O26:B6) was obtained from Sigma-Aldrich. For FACS analyses, mAbs against CD83 and CD86 were purchased from BD Pharmingen. Anti-DC-SIGN was obtained from R&D Systems. To characterize the viral surface, virus capture assays (VCA) were performed using anti-human C3c, C3d, IgG, and anti-mouse IgG1 mAbs (BD Pharmingen). For blocking experiments, a mAb against CD4 (clone SIM2; National Institutes of Health, Rockville, MD) was used. MIP-3β (CCL19) and secondary lymphoid tissue chemokine (CCL21) for migration assays were acquired from PeproTech. Monoclonal mouse Abs against CD81 and HLA-DM were obtained from BD Pharmingen. Anti-HLA-DR L-243 (a gift from J. Pieters, Biozentrum, University of Basel, Basel, Switzerland) and anti-CD63 (1B5; a gift from M. Marsh, Medical Research Council Cell Biology Unit and Department of Biochemistry and Molecular Biology, University College London, London, U.K.) were both used as supernatants of the corresponding hybridoma cells. The rabbit polyclonal anti-LAMP-1 was a gift from M. Fukuda (Cancer Research Center, La Jolla, CA) (30). Monoclonal mouse anti-HIV-1-p24^{gag} (KC57) was purchased from Coulter.

DC generation

DCs were generated as previously described by Wilflingseder et al. (31). FACS analysis was routinely used to confirm the purity (>95%) and the maturation stage of the cells. DCs for immunofluorescence and confocal microscopy were harvested at day 6, analyzed by flow cytometry, and used in subsequent assays. Additional technical details are available in Arrighi et al. (32) and Garcia et al. (33).

Preparation of HIV-IgGs and F(ab′)$_2$ of HIVIg

Sera from HIV-1-positive patients (provided by the Section of Serology, Innsbruck, Austria) were pooled and spun at 3300 rpm for 15 min. The serum pool was incubated for 2 h at 4°C with protein G-Sepharose (Amersham Biosciences). Bound IgG was eluted with 0.1 M glycine (pH 2.5), and eluted samples were immediately neutralized with 1 M Tris-buffered saline (pH 8.5). The protein concentration was determined at 280 nm and protein-containing fractions were pooled and dialyzed against PBS overnight (o/n). Ab concentration was again determined by photometry. F(ab′)$_2$ were prepared according to the manufacturer's instructions (Pierce).

Virus propagation and purification

Primary isolates 93BR020 (subtype B/F, X4/R5-tropic), 92UG001 (subtype D, X4/R5-tropic), 92BR030 (subtype B/B, R5-tropic), and 92UG037 (subtype A, R5-tropic) or the R5-tropic laboratory strain BaL were obtained by the National Institutes of Health-AIDS (available through World Health Organization depositories). Virus was propagated in PHA- and IL-2-stimulated PBMCs. Virus supernatants were cleared by filtration through 0.22-μm pore-size filters and concentrated by ultracentrifugation at 20,000 rpm for 90 min at 4°C. The virus pellet was resuspended in RPMI 1640 without supplements and stored in small aliquots at −80°C to avoid multiple thawing. One aliquot was taken to determine the virus concentration by p24 ELISA (34) and the 50% tissue culture infective dose of the viral stock. The endotoxin levels of the virus preparations used were below the detection limit of 0.005 U/ml (0.0005 ng/ml) and the threshold concentration for an LPS-induced effect on iDCs was determined to be 0.01 ng/ml (data not shown).

Opsonization of HIV-1

To mimic the in vivo situation, where HIV is opsonized with C-fragments, HIV-specific IgGs or a combination of both, the virus was incubated for 1 h at 37°C with normal human serum (NHS) as complement (C) source in a 1/10 dilution (C-HIV), with HIV-specific IgGs (5 μg/ml) to obtain IgG-opsonized virus (Ig-HIV), or a combination of both (C-Ig-HIV). The same procedure was performed to get F(ab′)$_2$-opsonized HIV-1 (FabIg-HIV) and NHS-F(ab′)$_2$ opsonized HIV-1 (C-FabIg-HIV). As negative control, the virus was incubated under the same conditions with RPMI 1640 medium or heat-inactivated serum (HIV). Sera from at least 10 healthy volunteers, referred to as NHS, were pooled and stored in small aliquots at −80°C until use. The presence of IgGs and C3 fragments on the viral surface was confirmed by a VCA as previously described (35).

Patient sera for opsonization

Heat-inactivated patient sera before (B) and after (A) seroconversion (nos. 1B-1A, 2B-2A, 3B-3A) were provided by the Section of Serology. The patient sera after seroconversion were used as Ab source for opsonization, those before seroconversion (no HIV-specific Abs) as controls. Opsonization of R5-tropic HIV-1-92UG037 with heat-inactivated patient sera before (1B, 2B, 3B) and after (1A, 2A, 3A) seroconversion was performed in the presence of NHS and the opsonization pattern was confirmed by VCA.

Infection of DCs and FcγRII-transfected HeLa-T4 cells

A total of 100 μl (1×10^5) DCs or FcγRII-transfected HeLa-T4 cells were incubated for 3 h at 37°C with 10 ng of p24/ml nonopsonized and opsonized virus preparations described above. Cells were extensively washed to remove unbound virus and resuspended in RPMI 1640/10% FCS/2 mM L-glutamine (RPMI$_c$) containing IL-4 (1000 U/ml) and GM-CSF (1600 U/ml) (DCs) or RPMI$_c$ (transfected HeLa-T4). Cells were cultivated at 37°C/5% CO_2 and supernatants were harvested at indicated time points.

In "classical" coculture experiments, 2×10^5 autologous nonstimulated T lymphocytes were added to 1×10^5 DCs. Classical coculture means that DCs were exposed to HIV for 3 h, washed, and T cells were added immediately. For infection experiments with T cells, the cells were stimulated for 2 days with PHA (2.5 μg/ml; Sigma-Aldrich) and IL-2 (100 U/ml) before infection. Virus concentrations from supernatants were determined by p24 ELISA on different days postinfection. Infection experiments were performed in triplicate and repeated five times with different HIV-1 strains. Because the infection efficiencies varied between the individual donors, figures in this article illustrate one representative experiment (plus SEM from the triplicates) and do not show the summarized data from all experiments.

Quantitative analyses of HIV-1 DNA dynamics by real-time PCR

Dynamics and integration efficiency of HIV-1 DNA in iDCs incubated for different periods of time with HIV, C-HIV, Ig-HIV, or C-Ig-HIV was assessed by real-time PCR using primers and fluorescently labeled probes for strong-stop HIV-1 DNA, or integrated forms of viral DNA as described by Suzuki et al. (36). SYBR Green was used for detection of the 2LTR DNA of HIV-1. Primers were slightly modified according to the sequence of the HIV-1 isolates used (BaL, 92UG037, 92UG001, and 92BR030) as indicated in Table I. The amount of integrated HIV-1 was determined by a nested PCR comprising an *Alu*-HIV-1 PCR as first step, followed by a second PCR using a dilution of the first cDNA products and the strong stop primers and probe set (Table I). A GAPDH PCR with GAPDH-specific primers and probe (Table I, italics) and a SYBR Green β-actin PCR served as controls to assess the relative quantity of the gene expression profile of the target genes (described below). PCRs using fluorescently labeled probes or SYBR Green were performed using the corresponding PCR kits of Stratagene according to the manufacturer's instructions. Thermal cycling conditions for GAPDH, β-actin, R/U5 (strong stop), and 2LTR PCR consisted of 10 min at 95°C and 50 cycles of 95°C for 15 s and 60°C for 30 s, and melt curve analyses for specification of PCR products in case of SYBR Green. The *Alu*-HIV-1 PCR was performed at 95°C for 10 min followed by 22 cycles of 30 s at 95°C, 30 s at 66°C, 10 min at 70°C, and a final extension step of 10 min at 72°C. All real-time PCR runs were performed on an iCycler (Bio-Rad).

Evaluation of HIV-1 DNA expression levels (strong stop, 2LTR, integrated)

The expression levels of the different HIV-1 DNA forms were calculated with the Gene Expression Macro software (version 1.1) from Bio-Rad. The calculations in this application allow the use of multiple reference genes. The calculations of the program are derived from the algorithms cited by

Table I. *Primers and probes for relative quantification of HIV-1 DNA expression levels*[a]

	Sense	Antisense	Probe
R/U5	5′-GGCTAGCTAGGGAACCCACTGC-3′	5′-CTGCTAGAGATTTTCCACACTGAC-3′ (BaL, 92UG001, 92BR030) 5′-GTGGTCTGAGGGATCTCTAGTT-3′ (92UG037)	5′F-TAGTGTGTGCCCGTCTGTTGTGTGAC-Q-3′
2LTR Int. DNA	5′-CTCAGACCCTTTTAGTCAGTGTGGAAAATC-3′	5′-TGACCCCTGGCCCTGGTGTAG-3′ AAAATC-3′	
First PCR	5′-TCCCAGCTACTCGGGAGGCTGAGG-3′	5′-CCTGCGTCGAGAGATCTCCTCTG-3′	
Second PCR	R/U5 sense	R/U5 antisense	R/U5 probe
GAPDH	5′-CTCATGACCACAGTCCATGC-3′	5′-CACGCCACAGTTTCCCG-3′	5′-F-CAGAAGACTGTGGATGGCCCC-Q-3′
Actin	5′-AGCCTCGCCTTTGCCGA-3′	5′-AGACGGCCGGCTCTGCAC-3′	

[a] F:FAM, Q:quencher, int., integrated.

Vandesompele et al. (37) and from the geNorm manual. The gene expression of the target genes (*R/U5*, *2LTR*, and *integrated HIV-1 DNA*) was quantified relatively by normalization with the reference genes *GAPDH* and *β-actin.* Uninfected iDCs were used as control samples to verify the specific amplification of the different HIV-1 DNA forms.

BDCA-1$^+$ (CD1c$^+$) DC isolation

Isolation of BDCA-1$^+$ (CD1c$^+$) DCs from blood of normal healthy donors was performed via magnetic beads according to the manufacturer's instructions (Miltenyi Biotec).

Immunofluorescence and confocal microscopy

To localize HIV-1, iDCs were pulsed with HIV-1 (500 ng of p24/2.5 $\times$ 10^5 cells) for 24 h, washed twice in PBS, and left to adhere on poly-L-lysine-treated (Sigma-Aldrich) glass coverslips for 1 h at 37°C. Cells were then fixed 20 min at room temperature in 3% paraformaldehyde, permeabilized with 0.05% saponin (Sigma-Aldrich), and washed with PBS containing 0.2% BSA (Sigma-Aldrich) and human IgG (20 μg/condition). Triple labeling of cells was done as follows: iDCs pulsed with HIV-1 were stained with primary Abs against CD81, HLA-DR, HLA-DM (all three monoclonal), CD63 (monoclonal (1B5), and LAMP-1 (polyclonal). After extensive washing in BSA/saponin-containing PBS, cells were then stained with secondary donkey anti-mouse and anti-rabbit Abs coupled to rhodamine or Cy-5, respectively (Jackson ImmunoResearch Laboratories). To avoid unspecific labeling, cells were incubated 20 min at room temperature in PBS containing BSA, saponin, and mouse serum (0.5 mg/ml). Finally, HIV-1-p24^{gag} was detected using a monoclonal anti-HIV-1-p24^{gag} (KC57) coupled to FITC. Confocal laser scanning microscopy was performed with a LSM 510 microscope (Zeiss). Images were then processed using Photoshop. Quantifications of colocalization were performed using the Metamorph software (Universal Imaging) on an average of 20–25 cells for each condition as previously described (33).

Analysis of FcγRIIA and FcγRIIB1/B2 expression in DCs by real-time RT-PCR

Expression of the ITAM-containing FcγRIIA and the ITIM-containing FcγRIIB1/B2 forms were analyzed in either iDCs or DCs treated for 48 h with LPS- or IgG-opsonized HIV using following primers: FcγRIIA, forward: 5′-CAGCATGGGCAGCTCTTC-3′ and reverse: 5′-CACATGGCA TAACGTTAC-3′ (38), FcγRIIB1/2, forward: 5′-GGTCACTGGGATTG CTGTAG-3′ (designed by Primer 3 Software) and reverse: 5′-CCCAAC TTTGTCAGCCTCATC-3′ (39). A SYBR Green GAPDH RT-PCR (Table I) was run simultaneously to perform relative quantification (see Fig. 6*A*). Cycling conditions were 30 min at 45°C to reverse transcribe the mRNA, 10 min at 95°C, 50 cycles of 15 s at 95°C, and 30 s at 60°C, and subsequent melt curve analysis to verify the specificity of the primers.

cDNA constructs

The FcγRIIB1 and FcγRIIB2 plasmids were cloned into the pRc/CMV vector (Invitrogen Life Technologies). FcγRIIA was inserted into an expression vector under the control of the SRα promoter in pBR322 (40), in which a resistance gene to zeocin was introduced (NT-zeo). All plasmids were provided by M. Daerön (Unité d'Allergologie Moléculaire et Cellulaire, Institut Pasteur, and Institut National de la Santé et de la Recherche Médicale Unite 760, Paris, France). Empty vectors served as negative controls.

Transfection of Hela-T4 cells

A total of 1 $\times$ 10^5 HeLa-T4 cells (Medical Research Council, London, U.K.) grown o/n at 37°C were transfected with the FcγRIIA, FcγRIIB1, the FcγRIIB2 plasmid or the empty vector as control using Metafectene (Biontex). The transfection efficiency was monitored by FACS analysis and was >70%.

Statistical analysis

Differences between triplicates in infection experiments and between the confocal microscopy samples were analyzed by GraphPad Prism software. Values of $p < 0.05$ in the unpaired Student t test were scored as significant.

Results

Opsonization of HIV with specific IgGs completely impairs infection of iDCs

We studied the capacity of the differentially opsonized virus preparations to productively infect iDCs. For this, iDCs were infected

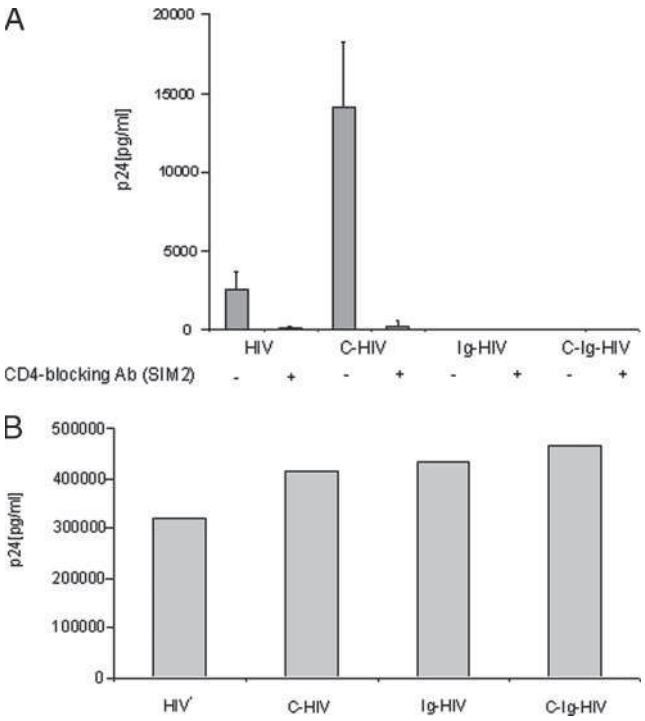

FIGURE 1. *A*, Infection of DCs by differentially opsonized HIV-1-preparations. iDCs were infected with differentially opsonized HIV (HIV, C-HIV, C-Ig-HIV, and Ig-HIV) in the absence and presence of SIM2, a CD4-blocking Ab. Supernatants (SNs) were taken for p24 measurement on several days postinfection (1, 5, 7, and 10 days). Nonopsonized and complement-coated HIV caused productive infection of DCs, while HIV incubated in the presence of specific IgGs did not productively infect the cells. SIM2 completely inhibited infection of DCs by HIV and C- HIV. The graph shows a representative infection experiment (day 10 postinfection) performed in triplicates of 10 independent experiments with different donors and different virus isolates. *B*, Infection efficiency in classical DC-T cell cocultures. iDCs were exposed to differentially opsonized HIV preparations for 3 h, cells were washed and nonstimulated T cells were added at a ratio 1:1. SNs for p24 measurement were taken on day 10 postinfection. All HIV preparations showed productive infection in cocultures to similar extends. The graph shows a representative infection experiment performed in triplicates of 10 independent experiments with different donors.

with 10 ng of p24/ml nonopsonized (HIV) or opsonized (C-HIV, Ig-HIV, and C-Ig-HIV), R5- (92UG037, 92BR030, and BaL), or dual-tropic (93BR020) HIV-1 preparations. As previously described (24, 41), iDCs were productively infected with nonopsonized and C-opsonized HIV (Fig. 1*A*, HIV, C-HIV). Opsonization of HIV with complement enhanced the infection of iDCs >3-fold compared with nonopsonized HIV (Fig. 1*A*). In contrast, virus that was opsonized with HIV-specific IgGs (Fig. 1*A*, Ig-HIV, C-Ig-HIV) showed no productive infection of iDCs. SIM2, a blocking anti-CD4 Ab, completely abolished infection of iDCs with both HIV and C- HIV (Fig. 1*A*). Nonproductive infection of DCs by IgG-opsonized HIV preparations was not due to the presence of neutralizing Abs in the HIV-IgG pool. This was excluded because all differentially opsonized HIV-1 preparations productively infected stimulated PBMCs (data not shown) and DC-T cell cocultures, in which T cells were added immediately (3 h) to HIV-exposed DCs (classical DC-T cell coculture) (Fig. 1*B*).

IgG opsonization of HIV impedes provirus formation in iDCs

To further characterize the differences in infection of iDCs between HIV, C-HIV, C-Ig-HIV, or Ig-HIV, a real-time PCR assay

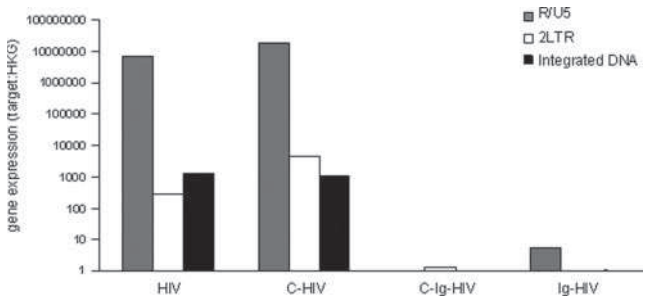

FIGURE 2. Provirus formation in DCs infected with differentially opsonized HIV. iDCs were exposed to 10 ng of p24/ml HIV, C-HIV, C-Ig-HIV, or Ig-HIV, cells were thoroughly washed after 3 h to remove unbound virus, and DNA was prepared 11 days after infection. Strong-stop (R/U5), 2LTR, and integrated DNA were detected in HIV- and C-HIV-exposed cells only, while integration of HIV was completely impaired in DCs loaded with C-Ig-HIV or Ig-HIV. This is a representative histogram of three experiments performed with different HIV isolates (BaL, 92BR030, and 92UG001).

for HIV-1 dynamics was applied. This assay should show simultaneously uptake and integration efficiency of nonopsonized and differentially opsonized virus preparations into iDCs. Thus, we analyzed DCs 11 days postexposure to HIV, C-HIV, C-Ig-HIV, or Ig-HIV by real-time PCR for strong stop (R/U5), 2LTR, and integrated viral DNA (Fig. 2). Only nonopsonized and C-opsonized HIV showed R/U5 and 2LTR transcripts (▨ and □, Fig. 2) and were efficiently integrated into genomes of iDCs (■, Fig. 2). In contrast, iDCs, which were loaded with IgG-opsonized HIV (C-Ig-HIV and Ig-HIV), did not complete integration and showed only very low levels of either 2LTR DNA (C-Ig-HIV, □, Fig. 2) or R/U5 DNA (Ig-HIV, ▨, Fig. 2). Measurement of p24 concentrations from supernatants of these DCs confirmed that only HIV- and C-HIV-loaded cells were productively infected with the virus, while no p24 was detectable in case of IgG-opsonized HIV (C-Ig-HIV and Ig-HIV) (data not shown). These results were verified for the laboratory strain BaL as well as for the primary isolates 92UG037 and 92BR030.

Our results of the DC infection assays, analyzed by p24 ELISA, coincide with real-time PCR data, demonstrating that both provirus formation and productive infection of DCs are completely impaired in iDCs exposed to HIV, which was opsonized in the presence of HIV-specific IgGs.

Ig-HIV is inefficiently transmitted to autologous T cells in long-term transfer experiments

Subsequent to infection experiments of iDCs with the differentially opsonized HIV preparations, migration of HIV-exposed DCs due to the CCR7 ligands MIP-3β and secondary lymphoid tissue chemokine were performed. Migration was already shown for DCs loaded with nonopsonized HIV (31). Migration of HIV-loaded DCs was not influenced by the opsonization pattern of the virus. iDCs exposed to HIV, C-HIV, Ig-HIV, or C-Ig-HIV migrated to similar extents in a CCR7-dependent manner. Additionally, migration of iDCs loaded with differentially opsonized HIV was not affected by the presence of the nucleoside reverse transcriptase inhibitor azidothymidine (data not shown). Next, we inquired whether migrated DCs transferred the differentially opsonized HIV-1 preparations to autologous, nonstimulated T cells. These experiments revealed that no or low transmission of IgG-coated virus from migrated DCs to $CD4^+$ cells was detectable (data not shown), while a vigorous infection was observed in classical DC-T cell cocultures with all differentially opsonized virus preparations (Fig. 1*B*).

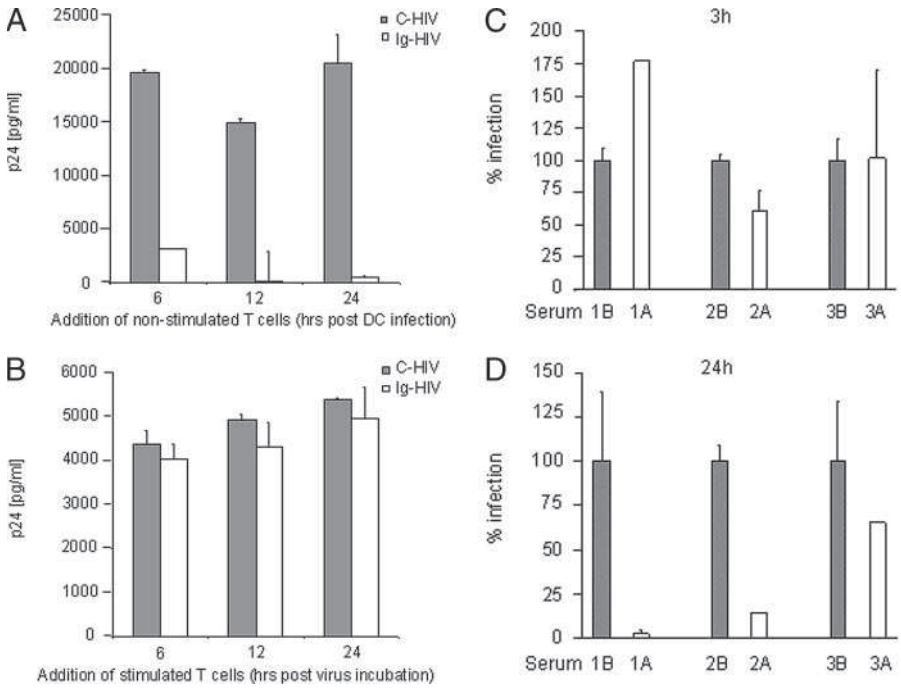

FIGURE 3. *A* and *B*, Long-term transmission of differentially opsonized HIV to T cells in the presence and absence of DCs. *A*, Ig-HIV is inefficiently transmitted from DCs to T cells in long-term experiments. Nonstimulated, autologous T cells were added to C-HIV- or Ig-HIV-loaded DCs after 6, 12, and 24 h. Whereas C-HIV caused vigorous infection at all time-points in DC-T cell cocultures, a strongly impaired infection with Ig-HIV was observed already after 6 h, which further decreased after 12 and 24 h. *B*, On the contrary to coculture experiments, stimulated T cells alone were infected with both HIV preparations (C-HIV and Ig-HIV) to the same extent at all time points. The experiments were performed twice in triplicates. *C* and *D*, Long-term transfer of HIV opsonized with sera from HIV-infected individuals before and after seroconversion. iDCs were loaded with HIV opsonized in the presence of sera from HIV-infected individuals obtained before seroconversion (1B, 2B, 3B) (no Abs) and after seroconversion (1A, 2A, 3A) (Ab source). Cells were washed and T cells were added either after short-term (3 h) (*C*) or long-term (24 h) incubation (*D*). The p24 concentration of the SNs was determined on day 7 postinfection. Again, the efficiency of infection was similar for all virus preparations, when T cells were added after 3 h, while in long-term experiments the transfer of Ab-coated HIV was impaired with all three sera tested. The figure shows a representative experiment performed in triplicates of three.

To figure out the kinetics of the impairment of infection in case of IgG-opsonized HIV in the presence of DCs, we added nonstimulated T cells 6, 12, and 24 h following loading of iDCs with either C- or Ig-HIV, respectively. In parallel control experiments, after 6, 12, and 24 h stimulated T cells were added to C-HIV and Ig-HIV, which were incubated in cell culture medium without DCs. Again, Ig-HIV showed a strongly impaired infection in DC-T cell cocultures already after incubation of DCs for 6 h, which further decreased after 12 and 24 h compared with C-HIV (Fig. 3*A*). By contrast, stimulated T cells were infected with C-HIV and Ig-HIV at all time points to similar extents in the absence of DCs (Fig. 3*B*).

Next, we investigated whether these differences were also observable, when the virus was opsonized with heat-inactivated sera from three different HIV-1-positive individuals before seroconversion and after seroconversion. For these experiments, the R5-tropic HIV primary isolate 92UG037 was opsonized in the presence of NHS as complement source together with either the heat-inactivated serum of the individual before seroconversion (i.e., no HIV-specific Abs) or the appropriate heat-inactivated serum after seroconversion (i.e., HIV-specific Abs). DCs were loaded with the different virus preparations for 3 h (i.e., short-term transfer) or o/n (i.e., long-term transfer) before addition of nonstimulated, autologous T cells. As shown in Fig. 3*C*, the infection efficiency in DC-T cell cocultures was similar for all virus preparations (1B-1A, 2B-2A, 3B-3A), when T cells were added to HIV-exposed DCs within 3 h. In contrast, delayed addition of T cells to Ig-HIV-loaded DCs (1A, 2A) caused a strongly impaired transmission and productive infection in two of the three individuals in comparison to DC-T cell cocultures infected with C-HIV (1B, 2B) (Fig. 3*D*). HIV opsonized with IgGs from the third individual (3A) showed an attenuated transmission and infection in long-term DC-T cell cocultures compared with C-HIV cocultures (3B) (Fig. 3*D*). But this decrease was not as pronounced as in the other two cases.

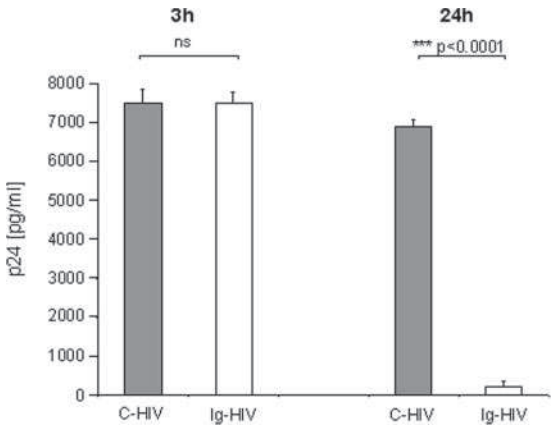

FIGURE 4. Transmission of differentially opsonized HIV from BDCA-1$^+$ DCs to T cells in long-term experiments. BDCA-1$^+$ DCs isolated directly from blood of healthy donors showed a significantly decreased transmission of Ig-HIV (□) in long-term experiments (24 h) compared with C-HIV (▨). In short-term experiments (3 h) the transmission efficiency was similar for C-HIV (▨) and Ig-HIV (□). The figure shows a representative experiment of four performed in triplicates.

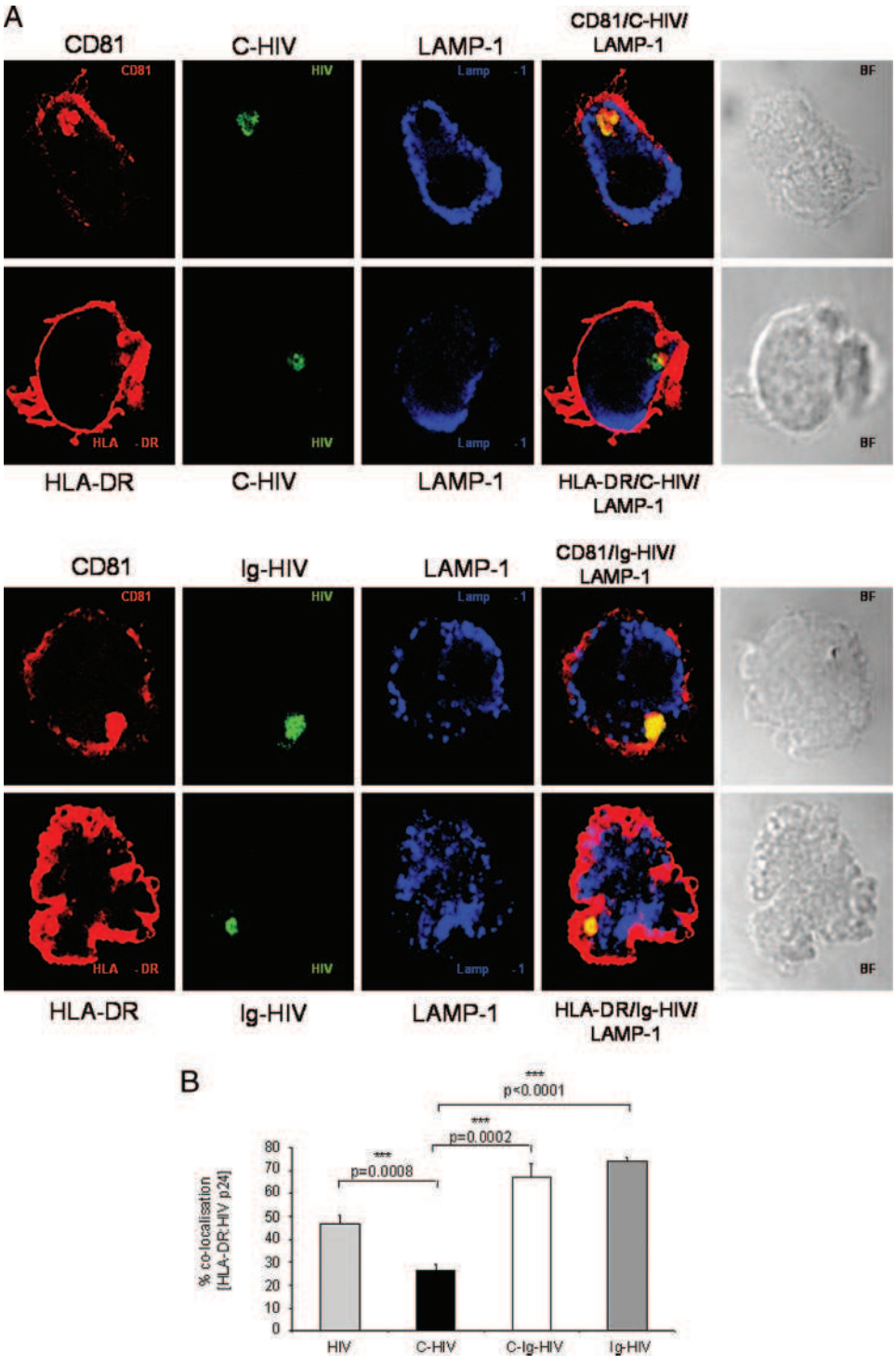

FIGURE 5. Analysis of intracellular compartments in DCs exposed to differentially opsonized HIV. iDCs were incubated for 24 h at 37°C with differentially opsonized HIV. *A*, iDCs loaded with HIV were analyzed by confocal immunofluorescence microscopy. One representative cell for C-HIV and Ig-HIV is depicted here with the corresponding cellular markers (green: immunostaining for HIV-1 $p24^{gag}$, red: cellular markers, blue: LAMP-1) Bar, 5 μm. *B*, The percentage of colocalization of differentially opsonized HIV preparations with HLA-DR is as shown here. The experiment was repeated three times and all the samples were scanned for at least 20–30 cells.

BDCA-1$^+$ blood DCs show decreased transmission of C-Ig-HIV and Ig-HIV to T cells

Circulating myeloid DCs were shown to coexpress activating FcγRs CD32a and CD64 (9, 42) and the inhibitory FcγR CD32b (9). We isolated BDCA-1$^+$ DCs directly from blood of healthy donors to confirm the results observed with in vitro-generated monocyte-derived DCs. Similar to monocyte-derived DCs, BDCA-1$^+$ DCs showed impaired transmission of Ig-HIV after delayed addition (24 h, $p < 0.0001$) of autologous, nonstimulated T cells compared with BDCA-1$^+$ DCs loaded with C-HIV (Fig. 4). Again, the same infection efficiency was observed when T cells were added immediately (3 h) to C-HIV- and Ig-HIV-loaded BDCA-1$^+$ DCs (Fig. 4).

HIV, C-HIV, C-Ig-HIV, and Ig-HIV behave differently with respect to HLA-DR association

To see whether the observed differences in iDCs are due to different internalization of the virus preparations, we next investigated the internalization pattern of nonopsonized HIV and differentially opsonized HIV in iDCs. The experiments were performed using the dual-tropic primary isolate 93BR020. iDCs were incubated with the various HIV preparations for 24 h at 37°C. Cells

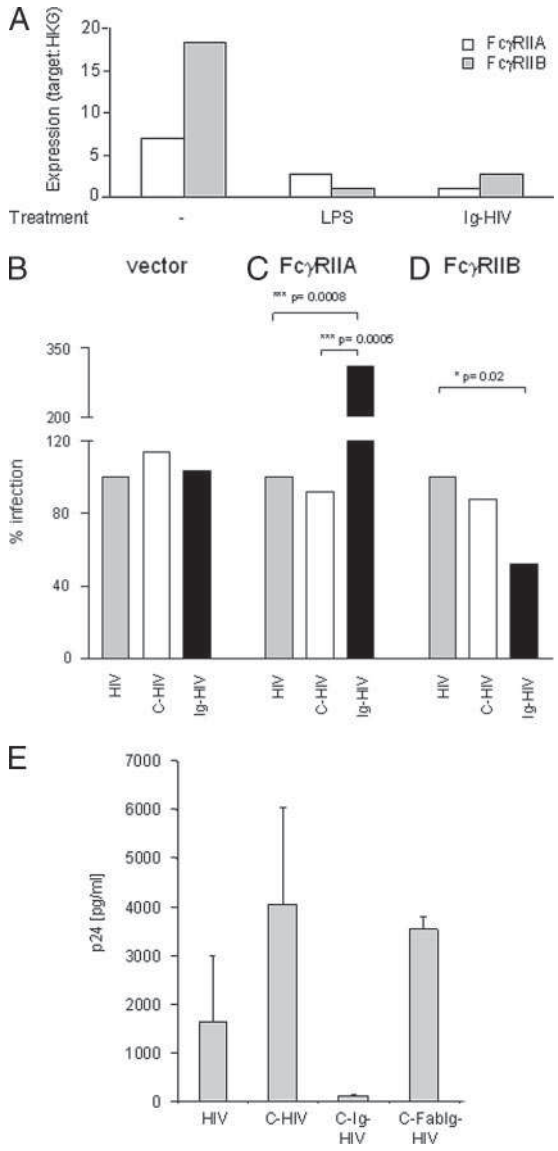

FIGURE 6. *A*, Expression of FcγRIIA and FcγRIIB in iDCs, LPS-treated DCs, and Ig-HI-exposed DCs. iDCs showed the highest expression of the stimulatory FcγRIIA and the inhibitory FcγRIIB. Treatment of iDCs with either LPS or IgG-opsonized-HIV for 48 h decreased the expression of both FcγRIIA and FcγRIIB. To relatively quantify the expression, a normalization to the HKG GAPDH was performed as described in *Materials and Methods*. *B–D*, Infection of control vector-, FcγRIIA-, and FcγRIIB-transfected HeLa-T4-cells with differentially opsonized HIV. *B*, Control vector-transfected HeLa-T4 cells showed similar infection with HIV, C-HIV, and Ig-HIV. *C*, A significantly higher infection of FcγRIIA-transfected HeLa-T4 cells was observed with Ig-HIV compared with HIV or C-HIV. *D*, In contrast, we observed a significantly lower infection of FcγRIIB-transfected HeLa-T4 cells with Ig-HIV compared with HIV or C-HIV. This is a representative of three independent infection experiments with the dual-tropic primary isolate 93BR020. *E*, Infection of monocyte-derived DCs with HIV, C-HIV, C-Ig-HIV, or C-FabIg-HIV. DCs were infected with 10 ng of p24/ml the differentially opsonized HIV-1 preparations and p24 ELISAs of the SNs taken on several days postinfection were performed. The figure shows a representative experiment performed in triplicates.

were then washed, allowed to adhere to coverslips, fixed, stained with appropriate Abs, and analyzed by immunofluorescence confocal microscopy (Fig. 5). Pixel analysis (data not shown) revealed that nonopsonized as well as differentially opsonized HIV targeted the same tetraspanin-rich compartment, characterized by the strong presence of CD81 (Fig. 5*A*, *upper file*, red). Colocalization with other cellular markers such as CD63 (marker of late endosomes/multi vesicular bodies) (data not shown), HLA-DM (data not shown), or Lamp-1 (marker of lysosomes) (Fig. 5*A*, blue) was marginal as previously published (33). Significant differences, particularly between C-HIV and IgG-opsonized HIV (C-Ig-HIV, Ig-HIV), were detected with respect to their HLA-DR colocalization in iDCs (Fig. 5*A*, lower line, red, and *B*). While HIV colocalized to ~50% with HLA-DR (Fig. 5*B*), C-HIV showed low colocalization with this marker (27.3, 25, and 31% in three different experiments; Fig. 5*B*). In contrast, C-Ig-HIV (71.4, 63.5, and 69%; Fig. 5*B*) and Ig-HIV (71.4, 76.2, and 70%; Fig. 5*B*) strongly colocalized with HLA-DR.

FcγRIIB-transfected cells show a decreased infection with IgG-opsonized HIV

Because both the activating and the inhibitory FcγRII receptors CD32a and CD32b were shown to be coexpressed on DCs (Boruchov et al. (9); Fig. 6*A*) and to better characterize the contribution of the activating CD32a and inhibitory CD32b1/b2 on the infection process when HIV is opsonized with HIV-specific IgGs, HeLa-T4 cells transfected with FcγRIIA, FcγRIIB, or an empty vector control were used for infection experiments. Cells were incubated with HIV, C-HIV, or Ig-HIV. We found that HIV and C-HIV productively infected vector-, FcγRIIA, and FcγRIIB-transfected HeLa-T4 cells to similar amounts (Fig. 6, *B–D*; ▨ and □). A highly significant enhancement of infection was observed when HeLa-T4 cells, transfected with the plasmid encoding the stimulatory FcγRIIA, were incubated with IgG-opsonized HIV (Fig. 6*C*, ■) compared with nonopsonized HIV or C-HIV, respectively.

In contrast, a significant reduction of productive infection of FcγRIIB1-transfected HeLa-T4 cells was measured when we used the IgG-opsonized virus preparation (Fig. 6*D*, ■) compared with nonopsonized HIV (52.4 vs 100%).

Infection of DCs is not impaired using F(ab′)$_2$ IgG-opsonized HIV

To further characterize the contribution of the inhibitory FcγRIIb expressed on DCs, we incubated the cells with F(ab′)$_2$ IgG-opsonized HIV in addition to the other differentially opsonized HIV-1 preparations. Although non-, C-, C-F(ab′)$_2$- (Fig. 6*E*), and F(ab′)$_2$-opsonized HIV (data not shown) caused productive infection of DCs, IgG-opsonized HIV (C-Ig-HIV (Fig. 6*E*), Ig-HIV (data not shown)) again showed a strongly impaired infection of the cells. These experiments let us suppose that the FcγRIIb plays an essential role in inhibiting DC infection with IgG-opsonized HIV and subsequent long-term transfer to T cells.

Discussion

The present study demonstrates that different opsonization patterns of HIV, detectable in HIV-1-infected individuals, have a profound impact on infection of and integration of monocyte-derived and circulating DCs with HIV-1. As reflected by efficient integration into DCs, nonopsonized and complement-opsonized HIV, which can be found at the acute phase of HIV pathogenesis, productively infected the cells. In contrast, no or a significantly impaired infection of and provirus formation in DCs was observed with HIV opsonized in the presence of HIV-specific IgGs independent of complement. The inhibition was not due to neutralizing Abs in the HIV-IgG pool because all differentially opsonized HIV preparations caused a vigorous infection in classical DC-T cell cocultures and cultures of stimulated T cells. In addition to nonproductive

infection of DCs, IgG-opsonization of HIV interfered with long-term transfer from DCs to T cells. This is in agreement with Turville et al. (43) as well as Burleigh et al. (44), who demonstrated that productive infection of DCs with HIV is a prerequisite for long-term transfer of the virus to T cells. Complement opsonization of HIV caused binding to CR-positive cells and enhanced the infection of these cells in "*cis*" and/or "*trans*." An increased infection due to complement opsonization of HIV was shown for macrophages, monocytes, or DCs (in *cis*) and DC-T cell or B cell-T cell cocultures (in *trans*) (20, 22, 24, 41, 45–49). Efficient transmission of infectious nonopsonized HIV from DCs to susceptible $CD4^+$ T cells was characterized in more detail by Turville et al. (43). A two-phase transfer of HIV from DCs to T cells is divided into the first phase of HIV transmission from 0 to 24 h, resulting from bypassing of the virus from endolysosomal compartments (*trans* infection), and the second phase from 24 to 72 h, resulting from "de novo" infection of DCs (*cis* infection) before transfer of HIV to T cells. These findings are in line with our results, because nonopsonized and complement-coated HIV emerged to be highly effective respecting short- (3 h, first phase) and long-term (24 h, second phase) transfer to nonstimulated, autologous $CD4^+$ T cells. Both virus preparations caused de novo productive infection of the DCs (in *cis*) and were efficiently integrated. In agreement with the idea that long-term transfer of HIV from DCs to T cells requires replication of virus in DCs, IgG-HIV-loaded DCs, which were not productively infected by the virus, were unable to promote long-term transmission to susceptible T cells. This observation turned out to be independent of the type of DCs (monocyte-derived or directly isolated BDCA-1^+ DCs) and also of the HIV-specific IgGs (pool or from a single donor after seroconversion, Fig. 3, *C* and *D*) used for the experiments.

Efficient inhibition of HIV infection in human DCs by purified anti-HIV-1 IgG was described very recently by Holl et al. (28, 29). They showed that purified polyclonal IgGs displayed a higher HIV-inhibitory capacity on monocyte-derived immature dendritic cells than on PHA-stimulated PBMCs. Furthermore, kinetics studies of Ab addition revealed that if neutralizing mAb 2F5 or neutralizing polyclonal Abs from HIV-infected individuals were added 3 h after HIV-loading of DCs, the HIV-inhibitory activity was lost. Thus, they assume that the anti-HIV Abs interfered with the first steps of virus entry into DCs. By analyzing the viral entry of differentially opsonized HIV preparations by real-time PCR at several shorter time points (2 and 5 h and 1, 2, and 5 days) after incubation of DCs, we provide evidence that HIV enters the cells independent of coating of the virus with HIV-specific IgGs. All differently opsonized HIV-1 preparations displayed high amounts of the strong-stop and the full-length transcript, but only nonopsonized and C-coated HIV showed the integrated HIV-1 DNA fragment (our unpublished data). Thus, both C-Ig-HIV and Ig-HIV enter DCs, but do not infect the cells. To identify the differences in provirus formation in DCs, we tracked intracellular virus using confocal microscopy. Dual-tropic HIV, C-HIV, C-Ig-HIV, and Ig-HIV accumulated in the tetraspanin-rich compartment as previously described for an HIV-X4 viral strain (33). Furthermore, we did not detect an accumulation of Ig-HIV in lysosomal compartments. Significant differences were observed with respect to HLA-DR colocalization. While C-Ig-HIV and Ig-HIV strongly colocalized with HLA-DR compartments, C-HIV colocalized to a much lower extend within these compartments. Recently, Finzi et al. (50) reported a role for HLA-DR on inducing Gag relocation to intracellular compartments harboring LE/MVB markers and found a substantial decrease in virus production and release from the cell surface of HLA-DR-expressing cells. This reduction was not detectable with HLA-DR molecules lacking their cytoplasmic domains, or with distinct MHC class II molecules such as HLA-DM or HLA-DO. In addition to the distinct HLA-DR/HIV colocalization, we cannot rule out more subtle differences between C-opsonized and IgG-opsonized virus, which have to be investigated.

Another candidate to explain the observed differences is the inhibitory FcγR CD32b (FcγRIIb). By using transfected cell lines for infection experiments and by analyzing the infection of DCs with $F(ab')_2$ IgG-opsonized HIV, we found that the inhibitory FcγRIIb may play a pivotal role in mediating the inhibitory effects observed with HIV-IgG-opsonized virus and DCs. Putative FcγRIIb interference with signaling pathways upon triggering with Ig-HIV needs to be determined. At the beginning of infection, nonopsonized HIV or C-HIV may interact with C-type lectins or CRs expressed on cells. After seroconversion, C-IgG- or IgG-opsonized HIV can in addition interact with FcγR-expressing cells. Both, monocyte-derived and BDCA-1^+ DCs coexpress CD32a and CD32b on their cell surface (9, 51, 52). Although culturing DCs in presence of 10% FCS decreases the frequency of FcγR expression (9), our experiments revealed that the density of FcγRs expressed on FCS-cultured iDCs was sufficient to exert the observed effects with the differentially opsonized HIV-1 preparations.

In summary, our data imply that dependent on the opsonization pattern, HIV exerts different modulatory effects on DCs, which might explain the low frequency of HIV-infected DCs found in vivo after seroconversion. The proposed role of DCs as reservoirs of HIV-1 that constantly contribute to infection of newly recruited T cells (53, 54) could hold true at the beginning of infection, when DCs are confronted with (non- or) complement-opsonized HIV in vivo. In vitro, these virus preparations have been shown to cause productive infection of and integration into DCs as well as effective short- and long-term transfer from DCs to T cells. In contrast, IgG opsonization of HIV was shown to impede DC infection, integration, and long-term transfer to susceptible T cells. Opsonization of HIV with specific IgGs might not interfere with transfer of virus from DCs to T cells, if the DC-T cell synapse is rapidly formed. Once a certain time frame exceeded between exposure of DCs to IgG-opsonized HIV and contact to T cells, the transfer to T cells is significantly impaired. Thus, understanding the exact mechanisms underlying the observed differences triggered in DCs by complement- and/or IgG-opsonized HIV at the level of extra- and intracellular events in vitro may in part explain the dysfunction of DCs described in HIV-positive individuals (25, 55).

Disclosures

The authors have no financial conflict of interest.

References

1. Banchereau, J., and R. M. Steinman. 1998. Dendritic cells and the control of immunity. *Nature* 392: 245.
2. Bánki, Z., L. Kacani, B. Müllauer, D. Wilflingseder, G. Obermoser, H. Niederegger, H. Schennach, G. M. Sprinzl, N. Sepp, A. Erdei, et al. 2003. Cross-linking of CD32 induce maturation of human monocyte-derived dendritic cells via NF-κB signalling pathway. *J. Immunol.* 170: 3963–3970.
3. Geijtenbeek, T. B., D. S. Kwon, R. Torensma, S. J. van Vliet, G. C. van Duijnhoven, J. Middel, I. L. Cornelissen, H. S. Nottet, V. N. KewalRamani, D. R. Littman, et al. 2000. DC-SIGN, a dendritic cell-specific HIV-1-binding protein that enhances trans-infection of T cells. *Cell* 100: 587–597.
4. Preynat-Seauve, O., C. L. Villiers, G. Jourdan, M. J. Richard, J. Plumas, A. Favier, P. N. Marche, and M. C. Favrot. 2004. An interaction between CD16 and CR3 enhances iC3b binding to CR3 but is lost during differentiation of monocytes into dendritic cells. *Eur. J. Immunol.* 34: 147–155.
5. Stahl, P. D., and R. A. Ezekowitz. 1998. The mannose receptor is a pattern recognition receptor involved in host defense. *Curr. Opin. Immunol.* 10: 50–55.
6. Akira, S., K. Takeda, and T. Kaisho. 2001. Toll-like receptors: critical proteins linking innate and acquired immunity. *Nat. Immunol.* 2: 675–680.
7. Stingl, G., E. C. Wolff-Schreiner, W. J. Pichler, F. Gschnait, W. Knapp, and K. Wolff. 1977. Epidermal Langerhans cells bear Fc and C3 receptors. *Nature* 268: 245–246.

8. Valladeau, J., V. Duvert-Frances, J. J. Pin, M. J. Kleijmeer, S. Ait-Yahia, O. Ravel, C. Vincent, F. Vega, Jr., A. Helms, D. Gorman, et al. 2001. Immature human dendritic cells express asialoglycoprotein receptor isoforms for efficient receptor-mediated endocytosis. *J. Immunol.* 167: 5767–5774.
9. Boruchov, A. M., G. Heller, M. C. Veri, E. Bovini, J. V. Ravetch, and J. W. Young. 2005. Activating and inhibitory IgG Fc receptors on human DCs mediate opposing functions. *J. Clin. Invest.* 115: 2914–2923.
10. Ravetch, J. V., and L. L. Lanier. 2000. Immune inhibitory receptors. *Science* 290: 84–89.
11. Greenberg, S., P. Chang, and S. C. Silverstein. 1993. Tyrosine phosphorylation is required for Fc receptor-mediated phagocytosis in mouse macrophages. *J. Exp. Med.* 177: 529–534.
12. Heath, S. L., J. G. Tew, A. K. Szakal, and G. F. Burton. 1995. Follicular dendritic cells and human immunodeficiency virus infectivity. *Nature* 377: 740–744.
13. Jakubik, J. J., M. Saifuddin, D. M. Takefman, and G. T. Spear. 2000. Immune complexes containing human immunodeficiency virus type 1 primary isolates bind to lymphoid tissue B lymphocytes and are infectious for T lymphocytes. *J. Virol.* 74: 552–555.
14. Hess, C., T. Klimkait, L. Schlapbach, V. Del Zenero, S. Sadallah, E. Horakova, G. Balestra, V. Werder, C. Schaefer, M. Battegay, and J. A. Schifferli. 2002. Association of a pool of HIV-1 with erythrocytes in vivo: a cohort study. *Lancet* 359: 2230–2234.
15. Banki, Z., L. Kacani, P. Rusert, M. Pruenster, D. Wilflingseder, B. Falkensammer, H. J. Stellbrink, J. van Lunzen, A. Trkola, M. P. Dierich, and H. Stoiber. 2005. Complement dependent trapping of infectious HIV in human lymphoid tissues. *AIDS* 19: 481–486.
16. Jouault, T., F. Chapuis, R. Olivier, C. Parravicini, E. Bahraoui, and J. C. Gluckman. 1989. HIV infection of monocytic cells: role of antibody-mediated virus binding to Fc-γ receptors. *AIDS* 3: 125–133.
17. Stoiber, H., A. Clivio, and M. P. Dierich. 1997. Role of complement in HIV infection. *Annu. Rev. Immunol.* 15: 649–674.
18. Döpper, S., H. Stoiber, L. Kacani, G. M. Sprinzl, F. Steindl, W. Prodinger, and M. P. Dierich. 2000. HIV-1 infection of allogenic and autologous primary T cells: complement-dependent and B cell-mediated enhancement. *Immunobiology* 202: 293–305.
19. Moir, S., A. Malaspina, Y. Li, T. W. Chun, T. Lowe, J. Adelsberger, M. Baseler, L. A. Ehler, S. Liu, R. T. Davey, Jr., et al. 2000. B cells of HIV-1-infected patients bind virions through CD21-complement interactions and transmit infectious virus to activated T cells. *J. Exp. Med.* 192: 637–646.
20. Stoiber, H., L. Kacani, C. Speth, R. Wurzner, and M. P. Dierich. 2001. The supportive role of complement in HIV pathogenesis. *Immunol. Rev.* 180: 168–176.
21. Smith-Franklin, B. A., B. F. Keele, J. G. Tew, S. Gartner, A. K. Szakal, J. D. Estes, T. C. Thacker, and G. F. Burton. 2002. Follicular dendritic cells and the persistence of HIV infectivity: the role of antibodies and Fcγ receptors. *J. Immunol.* 168: 2408–2414.
22. Doepper, S., D. Wilflingseder, W. Prodinger, G. Stiegler, M. P. Dierich, and H. Stoiber. 2003. Mechanism(s) promoting HIV-infection of primary unstimulated T lymphocytes in autologous B cell-T cell co-cultures. *Eur. J. Immunol.* 33: 2098–2107.
23. Takada, A., and Y. Kawaoka. 2003. Antibody-dependent enhancement of viral infection: molecular mechanisms and in vivo implications. *Rev. Med. Virol.* 13: 387–398.
24. Pruenster, M., D. Wilflingseder, Z. Bankí, C. G. Ammann, M. Meyer, M. P. Dierich, and H. Stoiber. 2005. C-type lectin independent interaction of opsonized HIV with monocyte-derived DCs. *Eur. J. Immunol.* 35: 2691–2698.
25. Donaghy, H., B. Gazzard, F. Gotch, and S. Patterson. 2003. Dysfunction and infection of freshly isolated blood myeloid and plasmacytoid dendritic cells in patients infected with HIV-1. *Blood* 101: 4505–4511.
26. Otero, M., G. Nunnari, D. Leto, J. Sullivan, F. X. Wang, I. Frank, Y. Xu, C. Patel, G. Dornadula, J. Kulkosky, and R. J. Pomerantz. 2003. Peripheral blood dendritic cells are not a major reservoir for HIV type 1 in infected individuals on virally suppressive HAART. *AIDS Res. Hum. Retroviruses* 19: 1097–1103.
27. Cameron, P. U., U. Forsum, H. Teppler, A. Granelli-Piperno, and R. M. Steinman. 1992. During HIV-1 infection most blood dendritic cells are not productively infected and can induce allogeneic $CD4^+$ T cells clonal expansion. *Clin. Exp. Immunol.* 88: 226–236.
28. Holl, V., M. Peressin, S. Schmidt, T. Decoville, S. Zolla-Pazner, A. M. Aubertin, and C. Moog. 2006a. Efficient inhibition of HIV-1 replication in human immature monocyte-derived dendritic cells by purified anti-HIV-1 IgG without induction of maturation. *Blood* 107: 4466–4474.
29. Holl, V., M Peressin, S. Schmidt, T. Decoville, S. Zolla-Pazner, A. M. Aubertin, and C. Moog. 2006. Nonneutralizing antibodies are able to inhibit human immunodeficiency virus type 1 replication in macrophages and immature dendritic cells. *J. Virol.* 80: 6177–6181.
30. Carlsson, S. R., J. Roth, F. Piller, and M. Fukuda. 1988. Isolation and characterization of human lysosomal membrane glycoproteins, h-lamp-1 and h-lamp-2: major sialoglycoproteins carrying polylactosaminoglycan. *J. Biol. Chem.* 263: 18911–18919.
31. Wilflingseder, D., B. Müllauer, H. Schramek, Z. Banki, M. Pruenster, M. P. Dierich, and H. Stoiber. 2004. HIV-1-induced migration of monocyte-derived dendritic cells is associated with differential activation of MAPK pathways. *J. Immunol.* 173: 7497–7505.
32. Arrighi, J. F., M. Pion, E. Garcia, J. M. Escola, Y. van Kooyk, T. B. Geijtenbeek, and V. Piguet. 2004. DC-SIGN-mediated infectious synapse formation enhances X4 HIV-1 transmission from dendritic cells to T cells. *J. Exp. Med.* 200: 1279–1288.
33. Garcia, E., M. Pion, A. Pelchen-Matthews, L. Collinson, J. F. Arrighi, G. Blot, F. Leuba, J. M. Escola, N. Demaurex, M. Marsh, and V. Piguet. 2005. HIV-1 trafficking to the dendritic cell-T-cell infectious synapse uses a pathway of tetraspanin sorting to the immunological synapse. *Traffic* 6: 488–501.
34. Purtscher, M., A. Trkola, G. Gruber, A. Buchacher, R. Predl, F. Steindl, C. Tauer, R. Berger, N. Barrett, A. Jungbauer, et al. 1994. A broadly neutralizing human monoclonal antibody against gp41 of human immunodeficiency virus type 1. *AIDS Res. Hum. Retroviruses* 10: 1651–1658.
35. Frank, I., H. Stoiber, S. Godar, J. Möst, H. Stockinger, and M. P. Dierich. 1996. Acquisition of host cell-surface-derived molecules by HIV-1. *AIDS* 10: 1611–1620.
36. Suzuki, Y., N. Misawa, C. Sato, H. Ebina, T. Masuda, N. Yamamoto, and Y. Koyanagi. 2003. Quantitative analysis of human immunodeficiency virus type 1 DNA dynamics by real-time PCR: integration efficiency in stimulated and unstimulated peripheral blood mononuclear cells. *Virus Genes* 27: 177–188.
37. Vandesompele, J., K. De Preter, F. Pattyn, B. Poppe, N. Van Roy, A. De Paepe, and F. Speleman. 2002. Accurate normalization of real-time quantitative RT-PCR data by geometric averaging of multiple internal control genes. *Genome Biol.* 3: RESEARCH0034.
38. Okayama, Y., D. D. Hagaman, M. Woolhiser, and D. D. Metcalfe. 2001. Further characterization of FcγRII and FcγRIII expression by cultured human mast cells. *Int. Arch. Allergy Immunol.* 124: 155–157.
39. Cassel, D. L., M. A. Keller, S. Surrey, E. Schwartz, A. D. Schreiber, E. F. Rappaport, and S. E. McKenzie. 1993. Differential expression of FcγRIIA, FcγRIIB and FcγRIIC in hematopoietic cells: analysis of transcripts. *Mol. Immunol.* 30: 451–460.
40. Takebe, Y., M. Seiki, J. Fujisawa, P. Hoy, K. Yokota, K. Arai, M. Yoshida, and N. Arai. 1988. SRα promoter: an efficient and versatile mammalian cDNA expression system composed of the simian virus 40 early promoter and the R-U5 segment of human T-cell leukemia virus type 1 long terminal repeat. *Mol. Cell. Biol.* 8: 466–472.
41. Bajtay, Z., C. Speth, A. Erdei, and M. P. Dierich. 2004. Productive HIV-1 infection of dendritic cells via complement receptor type 3 (CR3, CD11b/CD18). *J. Immunol.* 173: 4775–4778.
42. Fanger, N. A., K. Wardwell, L. Shen, T. F. Tedder, and P. M. Guyre. 1996. Type I (CD64) and type II (CD32) Fcγ receptor-mediated phagocytosis by human blood dendritic cells. *J. Immunol.* 157: 541–548.
43. Turville, S. G., J. J. Santos, I. Frank, P. U. Cameron, J. Wilkinson, M. Miranda-Saksena, J. Dable, H. Stossel, N. Romani, M. Piatak, Jr., et al. 2004. Immunodeficiency virus uptake, turnover, and 2-phase transfer in human dendritic cells. *Blood* 103: 2170–2179.
44. Burleigh, L., P. Y. Lozach, C. Schiffer, I. Staropoli, V. Pezo, F. Porrot, B. Canque, J. L. Virelizier, F. Arenzana-Seisdedos, and A. Amara. 2006. Infection of dendritic cells (DCs), not DC-SIGN-mediated internalization of human immunodeficiency virus, is required for long-term transfer of virus to T cells. *J. Virol.* 80: 2949–2957.
45. Frank, I., L. Kacani, H. Stoiber, H. Stossel, M. Spruth, F. Steindl, N. Romani, and M. P. Dierich. 1999. Human immunodeficiency virus type 1 derived from cocultures of immature dendritic cells with autologous T cells carries T-cell-specific molecules on its surface and is highly infectious. *J. Virol.* 73: 3449–3454.
46. Geijtenbeek, T. B., and Y. van Kooyk. 2003. DC-SIGN: a novel HIV receptor on DCs that mediates HIV-1 transmission. *Curr. Top. Microbiol. Immunol.* 276: 31–54.
47. Trumpfheller, C., C. G. Park, J. Finke, R. M. Steinman, and A. Granelli-Piperno. 2003. Cell type-dependent retention and transmission of HIV-1 by DC-SIGN. *Int. Immunol.* 15: 289–298.
48. Turville, S., J. Wilkinson, P. Cameron, J. Dable, and A. L. Cunningham. 2003. The role of dendritic cell C-type lectin receptors in HIV pathogenesis. *J. Leukocyte Biol.* 74: 710–718.
49. McDonald, D., L. Wu, S. M. Bohks, V. N. KewalRamani, D. Unutmaz, and T. J. Hope. 2003. Recruitment of HIV and its receptors to dendritic cell-T cell junctions. *Science* 300: 1295–1297.
50. Finzi, A., A. Brunet, Y. Xiao, J. Thibodeau, and E. A. Cohen. 2006. Major histocompatibility complex class II molecules promote human immunodeficiency virus type 1 assembly and budding to late endosomal/multivesicular body compartments. *J. Virol.* 80: 9789–9797.
51. Hunter, S., Z. K. Indik, M. K. Kim, M. D. Cauley, J. G. Park, and A. D. Schreiber. 1998. Inhibition of Fcγ receptor-mediated phagocytosis by a nonphagocytic Fcγ receptor. *Blood* 91: 1762–1768.
52. Bave, U., M. Magnusson, M. L. Eloranta, A. Perers, G. V. Alm, and L. Ronnblom. 2003. FcγRIIa is expressed on natural IFN-α-producing cells (plasmacytoid dendritic cells) and is required for the IFN-α production induced by apoptotic cells combined with lupus IgG. *J. Immunol.* 171: 3296–3302.
53. Cameron, P. U., P. S. Freudenthal, J. M. Barker, S. Gezelter, K. Inaba, and R. M. Steinman. 1992. Dendritic cells exposed to human immunodeficiency virus type-1 transmit a vigorous cytopathic infection to $CD4^+$ T cells. *Science* 257: 383–387.
54. McIlroy, D., B. Autran, R. Cheynier, S. Wain-Hobson, J. P. Clauvel, E. Oksenhendler, P. Debre, and A. Hosmalin. 1995. Infection frequency of dendritic cells and $CD4^+$ T lymphocytes in spleens of human immunodeficiency virus-positive patients. *J. Virol.* 69: 4737–4745.
55. Macatonia, S. E., R. Lau, S. Patterson, A. J. Pinching, and S. C. Knight. 1990. Dendritic cell infection, depletion and dysfunction in HIV infected individuals. *Immunology* 71: 38–45.