LFA-1 Is a Key Determinant for Preferential Infection of Memory CD4⁺ T Cells by Human Immunodeficiency Virus Type 1

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Memory CD4⁺ T cells are considered a stable latent reservoir for human immunodeficiency virus type 1 (HIV-1) and a barrier to eradication of this retroviral infection in patients under therapy. It has been shown that memory CD4⁺ T cells are preferentially infected with HIV-1, but the exact mechanism(s) responsible for this higher susceptibility remains obscure. Previous findings indicate that incorporation of host-derived intercellular adhesion molecule 1 (ICAM-1) in HIV-1 increases virus infectivity. To measure the putative involvement of virus-anchored ICAM-1 in the preferential infection of memory cells by HIV-1, quiescent and activated naive and memory T-cell subsets were exposed to isogenic virions either lacking or bearing ICAM-1. Memory CD4⁺ T cells were found to be more susceptible than naive CD4⁺ T cells to infection with ICAM-1-bearing virions, as exemplified by a more important virus replication, an increase in integrated viral DNA copies, and a more efficient entry process. Interactions between virus-associated host ICAM-1 and cell surface LFA-1 under a cluster formation seem to be responsible for the preferential HIV-1 infection of the memory cell subset. Altogether, these data shed light on a potential mechanism by which HIV-1 preferentially targets long-lived memory CD4⁺ T cells.

There is an extensive diversity displayed by CD4⁺ T cells in terms of phenotype, function, and anatomical distribution. This cellular subtype is heterogeneous and can be subdivided into naive (CD45RA⁺) and memory (CD45RO⁺) subsets (reviewed in reference 44). Naive T lymphocytes exit the thymus, enter into the bloodstream, and get in lymphoid tissues through high endothelial venules. They circulate in both compartments until they encounter their cognate antigen. Maintained in a G0 state, naive T cells require a stimulation of ~20 h from dendritic cells exposing the related antigen to be committed to proliferate (26). Depending on the duration of T-cell receptor stimulation mediated by dendritic cells in combination with some cytokines, the activated T cells differentiate and reach distinct effector functions and homing and survival capacity. Cells receiving a weak stimulation die by apoptosis, whereas those receiving a strong stimulation become effector or enter the memory pool. The memory subset remains in a nondividing state (quiescent), expresses lymph node homing receptors, and has a higher sensitivity to antigenic stimulation compared to the naive one. Enhanced expression of adhesion molecules and cellular factors is mainly involved in their ability to rapidly undergo terminal differentiation upon exposure to a recall antigen (32, 46).

Although human immunodeficiency virus type 1 (HIV-1) replicates predominantly in activated CD4⁺ T lymphocytes (35), quiescent CD4⁺ T cells likely represent the major target for initial infection among T cells. Indeed, most T cells in the body are in a quiescent G₀ state with a low metabolic rate. However, numerous studies have reported that quiescent T cells are mainly nonpermissive to HIV-1 replication. In spite of this, integrated HIV-1 DNA has been largely found in resting CD4⁺ T lymphocytes from infected individuals (7, 16, 39). Interestingly, the majority of quiescent T cells carrying integrated HIV-1 genome displays a memory phenotype (10). While it is recognized that memory CD4⁺ T cells constitute the main cellular reservoir for HIV-1, it remains unclear whether these cells are more susceptible to the initial steps of viral life cycle.

Based on comparable surface levels of both primary cellular receptor and coreceptor (i.e., CD4 and CXCR4) on naive and memory T lymphocytes (43), it is usually thought that entry of HIV-1 occurs at similar rates in these two distinct cell subsets. However, besides interactions between the external virus-encoded envelope glycoprotein gp120 and CD4/CXCR4, it has been recognized that other interactions can promote the initial events in HIV-1 replication. Indeed, convincing studies have revealed that HIV-1 incorporates a plethora of host-derived cell surface molecules during the budding process, including the intercellular adhesion molecule 1 (ICAM-1) (9, 21, 57). Interestingly, the adhesion molecule ICAM-1 is efficiently acquired by all tested laboratory and clinical variants of HIV-1 bearing different tropisms (i.e., R5, X4, and R5X4) once amplified either in established cell lines or in primary human cells (2, 9, 12, 14, 23, 36). Moreover, it has also been demonstrated that virus-associated ICAM-1 influences HIV-1 biology since the natural ability of ICAM-1 to associate with its natural counterligand LFA-1 is preserved and leads to a severalfold increase in virus infectivity (21, 22, 55). Such a significant enhancement of HIV-1 infectivity is due to a more efficient virus adsorption onto target cells and a preferential entry process by fusion rather than through endocytosis (16).

Considering that memory CD4⁺ T cells express a higher surface level of LFA-1 compared to the naive subset, we tested...
the possibility that they might be preferentially targeted by ICAM-1-bearing HIV-1 particles. Our data indicate that mem-
ory CD4⁺ T lymphocytes are indeed more susceptible to a pro-
ductive HIV-1 infection than the naïve population, a pro-
cess that seems to be due partly to the presence of LFA-1
clusters. This study provides novel insights on the identity of
null cells, which is particularly relevant for understanding the
immune response to HIV-1.

MATERIALS AND METHODS

Antibodies and reagents. The anti-CD11a (clone MEM25) monoclonal anti-
body was purchased from EXBIO Praha (Vestec, Czech Republic), whereas the anti-
CD18 (CTB04) monoclonal antibody was purchased from Santa Cruz Bio-
technology, Inc. (Santa Cruz, CA). The hybridoma cell line that produces the
anti-CD4 SIM2 monoclonal antibody was provided by the AIDS Repository
Reagent Program (Germantown, MD). Anti-CD45RA (clone SH9) and
anti-CD45RO (clone UCHL-1) were purchased from BD Pharmingen (Mississauga,
Ontario, Canada). R-phycocerythin (R-PE)-conjugated and fluorescein isoi-
cyanate-tagged goat anti-mouse immunoglobulin G (IgG) were purchased from
Jackson ImmunoResearch Laboratories (West Grove, PA). The following anti-
bodies were obtained from Molecular Probes (Eugene, OR): Cy5 goat anti-
mouse IgG, Alexa Fluor 488 goat anti-human IgG, and Alexa Fluor 546 goat
anti-human IgG. Azide-thymidine (AZT) was purchased from Sigma-Aldrich
(Oakville, Ontario, Canada). The fusion inhibitor T-20 was provided by Roche
Bioscience (Palo Alto, CA), while lovastatin and pravastatin were purchased from
Calbiochem (San Diego, CA).

Cells. 293T cells were provided by W. C. Greene (The J. Gludstone Institutes,
San Francisco, CA). These cells were cultured in Dulbecco's modified Eagle's
medium (DMEM) supplemented with 10% fetal bovine serum (FBS). Peripheral
blood mononuclear cells (PBMCs) from healthy donors were isolated by Ficoll-
Hypaque gradient centrifugation, and CD4⁺ T cells were purified from fresh
isolated PBMCs by immunomagnetic negative selection as indicated by the manufacturer
(Stem Cell Technologies Inc., Vancouver, British Columbia, Can-
da). Purified CD4⁺ cells were further separated into pure CD45RA and
CD45RO populations by negative selection as described by the manufacturer
(Milenyi Biotec Inc., Auburn, CA). The purity of the T-cell subsets was deter-
mamed by cytofluorometric analyses and was always ≥98%. Lymphocytes were
cultured for 24 h in RPMI 1640 medium supplemented with 10% FBS in the
absence or presence of phytohemagglutinin (PHA; 1 μg/ml) and recombinant
human interleukin-2 (rIL-2; 1–50 U/ml).

Production and purification of viral stocks. pNL4-3 is a full-length infectious
molecular clone of HIV-1 (provided by the AIDS Repository Reagent Program).
pNL4ENG1-EGFP is a full-length infectious molecular clone of HIV-1 containing
isoenic HIV-1 particles, either lacking or bearing host-derived ICAM-1 (2.5 ng
of p24 per 10⁶ cells), and were incubated at 37°C for 1 or 3 h. To monitor the
role played by LFA-1 in the process of virus entry, cells were pretreated for
30 min at 37°C with an antibody known to abolish ICAM-1/LFA-1 interactions
(i.e., MEM25) before addition of viruses. In some experiments, T-cell subsets
were pretreated with an anti-CD4 antibody (i.e., 5 μg of p24/ml) for 30 min at 37°C
to abrogate interactions between gp120 and CD4 while virus fusion was blocked
by using the fusion inhibitor T-20 (20 μg/ml). Following virus exposure, cells
were washed and trypsinized for 5 min at 37°C to remove noninternalized viruses.
Next, cells were first washed once with RPMI-1640 supplemented with 10% FBS
and then twice with PBS before lysis in 200 μl of ice-cold lysis buffer (20 mM
HEPES [pH 7.4], 150 mM NaCl, 0.5% Triton X-100). The amount of viruses
entering cells was estimated by the p24 assay.

Detection of integrated HIV-1 DNA by real-time PCR. Naive and memory
CD4⁺ T cells were infected with isogenic NL4-3 or NL4-3-ICAM-1 particles for
3 days before extraction of genomic DNA by using the QIAGEN kit (Missis-
sauga, Ontario, Canada). DNA was quantified and subjected to a combined
Alu-HIV-1 PCR and real-time PCR as described by Suzuki and coworkers (54).
Briefly, genomic DNA (100 ng) was first amplified with an Alu-sequence-specific
sense primer (16) and HIV-1-specific antisense primer (i.e., M661). Next, the
PCR products were diluted to a 1:20 ratio and subjected to a standard PCR assay
with the sense primer M667, the antisense primer AA55, and the TaqMan probe
HIV-6-carboxyfluorescein (54, 55). The cycling conditions included a hot start
(95°C for 5 min), followed by 40 cycles of denaturation (95°C for 1 min) and
extension (63°C for 1 min). NL4-3 DNA was used for the standard curve (i.e.,
from 938 to 60,000 copies). HIV-1 standards contain 1 ng of DNA from un-
fected cells as carrier. Real-time PCR was performed by Rotor Gene RG-3000
(Corbett Research, distributed by Montreal Biotech Inc., Montreal, Quebec,
Canada).

Infecitivity analysis. Quiescent and activated T-cell subsets (10⁵ cells) were
incubated for 2 h at 37°C with isogenic NL4-3 or NL4-3-ICAM-1 virions (2.5
ng of p24). Cells were then extensively washed with PBS and resuspended in 200 μl
of culture medium supplemented with rIL-2. Cells were then transferred into
96-well plates (bottom tissue culture plates and incubated at 37°C; 5% CO₂). For experi-
ments, cells were treated with 10 μM AZT after an incubation of 4 h with
viruses. Following an additional incubation time period of 16 h, cells were
washed with culture medium and resuspended in 200 μl of culture medium
supplemented with rIL-2. Supernatants (100 μl) were harvested at different time
points and frozen at −20°C until assayed for p24 contents.

Separation of naive and memory CD4⁺ T cells following exposure to HIV-1.
Quiescent CD4⁺ T lymphocytes (2 × 10⁶ cells) were incubated for 6 h at 37°C
with HIV-1 particles either lacking or bearing host-derived ICAM-1 (5 ng of p24
per 10⁶ cells in a final volume of 0.5 ml of culture medium). In some experiments,
cells were pretreated with lovastatin (50 μM) for 30 min at 37°C to block the
interaction between LFA-1 and ICAM-1 or with the control compound prava-
statin (50 μM) before exposure to viruses. Cells were washed twice with ice-cold
PBS-2% FBS (binding buffer) and next incubated with magnetic beads to
achieve isolation of cell subpopulations (Dynabeads M-450; Dynal Biotech Inc.,
Brown Deer, WI). Briefly, magnetic beads (1.5 μl per assay) were first coated
with an anti-CD45RA or anti-CD45RO (1 ng per assay) antibody for 1 h at room
temperature on a rotating plate. Beads were next washed twice with binding
buffer through the use of a magnetic separation unit and resuspended in 100 μl
of binding buffer. Beads were finally washed twice with binding buffer with a
magnetic separation unit. Naive and memory quiescent CD4⁺ T lymphocytes,
which were positively selected, were resuspended in 400 μl of culture medium
containing PHA and IL-2 and incubated at 37°C. Supernatants were harvested

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cells were made. Indeed, resting memory CD4 T cells, observations similar to the ones obtained with activated subsets. Even though virus production was significantly re-

-ICAM-1 on the infection of quiescent naïve and memory T-cell donors. Next, we analyzed the impact of virus-associated not representative since we have not observed it with other infected with HIV-1 lacking the adhesion molecule, this result is comparable when using virions lacking ICAM-1 (Fig. 1A). Interestingly, a more massive virus production in the memory subset compared to the naive subpopulation was seen following infection with isogenic ICAM-1-bearing HIV-1 particles. Al-

to real-time PCR using primers specific for HIV-1 R/U5 re-
PCR amplification using primers specific for 

RESULTS

To define the possible contribution of virus-anchored host ICAM-1 in the reported ability of HIV-1 to replicate preferentially within memory CD4 + T lymphocytes, we produced isogenic HIV-1 particles (NL4-3 strain/X4 tropic) differing only by the absence or presence of host-derived ICAM-1 using a well-established transient transfection-and-expression system (21). The efficient incorporation of ICAM-1 onto progeny virus was monitored by a previously described virus capture assay (data not shown) (13).

Susceptibility of quiescent and activated CD4 + T-cell subsets to HIV-1 infection. Previous studies have clearly demonstrated that interactions between virus-associated ICAM-1 and cell surface LFA-1 play a dominant role in the initial steps of HIV-1 life cycle once infection is allowed to proceed in PHA/IL-2-activated CD4 + T lymphocytes (9, 21, 41). However, given that CD4 + T cells are heterogeneous both functionally and phenotypically, we were interested in monitoring whether the insertion of host ICAM-1 within HIV-1 can direct infection toward a specific cellular subset. Data from studies on HIV-1 replication kinetics demonstrated that levels of virus replication in activated naive and memory CD4 + T cells were comparable when using virions lacking ICAM-1 (Fig. 1A). Interestingly, a more massive virus production in the memory subset compared to the naive subpopulation was seen following infection with isogenic ICAM-1-bearing HIV-1 particles. Although Fig. 1A shows that the infection of activated naïve T cells with ICAM-1-bearing virions is lower than the ones infected with HIV-1 lacking the adhesion molecule, this result is not representative since we have not observed it with other donors. Next, we analyzed the impact of virus-associated ICAM-1 on the infection of quiescent naive and memory T-cell subsets. Even though virus production was significantly reduced when infections were carried out in quiescent CD4 + T cells, observations similar to the ones obtained with activated cells were made. Indeed, resting memory CD4 + T lymphocytes produced an average of 3.4-fold more viruses than did the naive subpopulation at 6 days postinfection (Fig. 1B).

Upon infection with ICAM-1-bearing virions, memory CD4 + T cells in a resting state carry more integrated HIV-1 DNA than the naive subset. Integration represents an essential step in the replicative cycle of retroviruses (19, 34, 62). It is generally considered that reverse transcription is impaired in naïve CD4 + T cells and that no integration can be detected in vitro. Nevertheless, it has been shown that, in HIV-1-seropositive patients, both quiescent naive and memory CD4 + T lymphocytes carry integrated proviral DNA that can support virus replication (39). Thus, we next estimated the levels of integrated HIV-1 DNA in quiescent naive and memory CD4 + T cells. This goal was achieved by performing a first round of PCR amplification using primers specific for Alu and HIV-1 sequences. The first PCR product was subsequently subjected to real-time PCR using primers specific for HIV-1 R/U5 regions. The Alu sequence is a ubiquitous repeat element found in the human genome (about 10 6 copies). By amplifying the junction between the nearest Alu sequence and the viral long terminal repeat (LTR), followed by a second round of LTR amplification, the presence of integrated HIV-1 genome can be specifically demonstrated. As shown in Fig. 2, the numbers of integrated viral DNA copies per ng of total DNA were comparable in resting naive (6,343 ± 1,987) and memory
since memory CD4+ T cells were incubated with virus preparations for 6 h at 37°C, washed, and maintained in culture for an additional 72 h. Genomic DNA was extracted and subjected in duplicate to a first PCR with Alu and M661 primers to amplify integrated proviruses. Next, the first PCR products were diluted 25-fold and subjected to real-time PCR using AA55 and M667 primers to further amplify the integrated HIV-1 LTR. The number of NL4-3 copies was determined by a standard curve prepared with the NL4-3 vector. Results shown are representative of two experiments made with different donors.

(11,218 ± 7,027) CD4+ T cells following infection with NL4-3 lacking host ICAM-1 as well as in quiescent naive CD4+ T cells inoculated with virions bearing the adhesion molecule (2,781 ± 3,932). However, a significant increase in the levels of integrated viral DNA was detected in memory CD4+ T cells that were infected with NL4-3/ICAM-1 particles (i.e., a mean increase of 22-fold; 254,000 ± 138,592). These data were confirmed when samples from a second healthy donor were used, since memory CD4+ T cells infected with ICAM-1-bearing viruses carried about 14-fold more integrated HIV-1 DNA copies than did the three other samples tested (data not shown).

Reverse transcription of ICAM-1-bearing virions is more efficient in memory CD4+ T cells. Following virus-cell fusion and uncoating, HIV-1 RNA undergoes reverse transcription to a DNA intermediate that can remain under either a nonintegrated or an integrated form. It has been recently suggested that the nonintegrated HIV-1 DNA can serve as a template for viral gene expression and has the full capacity to produce all classes of viral transcripts (50, 51, 63). However, it was shown that reverse transcription of the HIV-1 genome in quiescent T cells remains incomplete. Thus, if we detect the integrated HIV-1 genome in memory CD4+ T cells infected with NL4-3/ICAM-1, it would imply a more efficient reverse transcription process in this particular cell subset. To address this question, naive and memory CD4+ T cells were incubated with the studied virus preparations (i.e., NL4-3 and NL4-3/ICAM-1) for a time period sufficient to allow the binding, entry, and formation of partially or completely reverse-transcribed DNA before the addition of AZT (i.e., 4 h). Treatment with AZT will impair DNA synthesis from partially reverse-transcribed forms of viral genomic RNA, but not from completely synthesized viral DNA. Studies performed with this experimental strategy indicated that the release of progeny virus was observed only in AZT-treated memory CD4+ T cells that were infected with ICAM-1-bearing virions but not in the other samples subjected to AZT treatment (Fig. 3).

Higher levels of LFA-1 clusters are found on memory compared to naive CD4+ T cells. Reorganization of LFA-1 molecules into clusters represents one of the major mechanisms that regulate ICAM-1 binding (18, 31, 59–61). In naive CD4+ T cells, LFA-1 is maintained in a low avidity state (45, 48). Cellular activation favors lateral diffusion of the integrin subunit αLβ2 and formation of LFA-1 clusters (18, 28, 52, 64). Since memory CD4+ T cells originate from activated naive CD4+ T cells, on which LFA-1 molecules are found in clusters, we wanted to investigate whether this patching is preserved and can play a role in the observed higher susceptibility of memory CD4+ T cells to a productive infection with ICAM-1-bearing virions. FACS analyses demonstrate that all naive and memory CD4+ T cells express LFA-1 subunits (CD11a and CD18) but at different levels (Fig. 4A). The mean fluorescence intensities (MFI) of CD11a and CD18 are higher on the memory subset (blue line) than on naive ones (red line), as was previously demonstrated by others groups (reviewed in references 4 and 11). Distinct spatial distribution of LFA-1 on naive and memory T cells is observed when performing confocal microscopy analyses. Indeed, a weak but homogenous distribution of LFA-1 was found on the surface of naive CD4+ T cells (Fig. 4B). Interestingly, LFA-1 was localized in patches on memory CD4+ T lymphocytes in a quiescent state as well as on the two studied activated T-cell subpopulations. These results suggest that LFA-1 is distributed in clusters onto resting memory CD4+ T cells. To compare densities of LFA-1 clusters among the different CD4+ T-cell subsets, cells were stained with PHA for 24 and 48 h prior to analysis by flow cytometry. Results depicted in Fig. 4C demonstrate that levels of LFA-1 clusters are only marginally augmented following activation of
naive CD4\(^+\) T lymphocytes, which is in sharp contrast to the situation seen with the activated memory cell subset.

**ICAM-1-bearing viruses attach to and enter more efficiently in memory compared to naive CD4\(^+\) T cells.** Given that higher amounts of integrated HIV-1 DNA are found in memory CD4\(^+\) T lymphocytes, we addressed the possibility that the entry of ICAM-1-bearing virions is more efficient in this cellular subtype. To this end, entry of the studied virus preparations in purified CD4\(^+\)-T-cell subsets was estimated in the absence or presence of an anti-LFA-1 antibody that can abrogate ICAM-1/LFA-1 interactions (MEM25). The process of entry of viruses lacking ICAM-1 was not affected by the anti-LFA-1 antibody, and slight differences were detected between naive and memory CD4\(^+\) T cells (Fig. 5A). On the contrary, larger amounts of isogenic ICAM-1-bearing virions were found inside memory CD4\(^+\) T cells than in cells bearing the naive phenotype (4.7-fold increase). The contribution of ICAM-1-LFA-1 interactions in the observed enhancement of virus entry is clearly illustrated through the use of the LFA-1 neutralizing antibody. Cellular activation of both naive and memory CD4\(^+\) T cells led to an increase in the rate of entry of NL4-3/ICAM-1, suggesting that enhanced activation of LFA-1 as well as LFA-1 expression might be responsible for this phenomenon. Differences observed in the entry of ICAM-1-bearing virions into activated naive and memory T cells are linked to the levels of LFA-1 clusters. Indeed, resting memory and activated naive CD4\(^+\) T cells (24 h poststimulation) contain similar amounts of LFA-1 clusters (Fig. 4C). Interestingly, similar amounts of ICAM-1-bearing viruses are detected inside these cell subpopulations. In contrast, the amount of virions detected within activated memory CD4\(^+\) T lymphocytes is the largest among samples tested, a phenomenon associated with the more important level of LFA-1 clusters observed on these cells. Next, we assessed the contribution of CD4 and CXCR4 in entry of the studied virus preparations in resting naive and memory CD4\(^+\) T cells. We found that the entry of virions lacking ICAM-1 in naive and memory CD4\(^+\) T cells is weakly affected by the anti-CD4 antibody SIM.2 and the fusion inhibitor T-20 (Fig. 5B). These data suggest that, at least under the tested experimental conditions (i.e., virus entry monitored after 3 h following infection), virions lacking host ICAM-1 are mainly internalized by endocytosis and not by fusion in the studied target cells. Similar results were obtained when assessing the entry of ICAM-1-bearing virions inside resting naive CD4\(^+\) T cells. In contrast, entry of ICAM-1-bearing viruses within resting memory CD4\(^+\) T cells was found to be sensitive to the two blocking agents studied. These results are in agreement with our previous work demonstrating that ICAM-1 incorporation favors the entry of HIV-1 particles through fusion, a process known to result in productive infection (55, 56).

Confocal microscopy studies were next carried out to provide additional evidence of the capacity of ICAM-1-bearing HIV-1 particles to interact more efficiently with CD4\(^+\) T cells compared to the naive subset. As depicted in Fig. 5C, similar low levels of virions lacking host ICAM-1 were found to associate with both CD4\(^+\) T-cell subsets. A larger amount of NL4-3/ICAM-1 viruses was found to associate with resting memory CD4\(^+\) T lymphocytes than with naive CD4\(^+\) T cells. Activated memory CD4\(^+\) T cells, when used in combination with ICAM-1-bearing HIV-1 particles, were used as a positive control. The data further confirm that HIV-1 particles carrying ICAM-1 display a greater propensity to target memory CD4\(^+\) T cells.

**Cells of the memory phenotype in a heterogeneous resting CD4\(^+\)-T-cell population are preferentially targeted by ICAM-1-bearing virions.** In an attempt to assess if ICAM-1-bearing virions could preferentially target memory CD4\(^+\) T cells when infection is performed in unseparated CD4\(^+\) T lymphocytes, preparations of negatively selected CD4\(^+\) T lymphocytes were exposed to HIV-1 particles either lacking or bearing host ICAM-1. Cells were next separated using anti-CD45RA and anti-CD45RO-coated magnetic beads. Each population was maintained in culture medium containing PHA and IL-2 for 3 days, and supernatants were harvested to quantify the virus content. In some conditions, cells were pretreated either with lovastatin to inhibit interactions between ICAM-1 and LFA-1 interaction or with a control compound (i.e., pravastatin). Results presented in Fig. 6 indicate that virions lacking host ICAM-1 infected both naive and memory CD4\(^+\) T cells with a comparable low efficiency. However, memory CD4\(^+\) T cells were more efficiently infected with ICAM-1-bearing viruses than cells expressing the naive phenotype, a phenomenon that was abolished upon treatment with lovastatin but not with pravastatin.

**DISCUSSION**

Identification of intrinsic factors responsible for the preferential infection of memory cells among resting CD4\(^+\) T cells is of high importance to achieve a better comprehension of HIV-1 biology. This knowledge is particularly informative since it might help to shed light on mechanisms through which viral reservoirs are established in infected individuals and on the inability of the immune system to better control HIV-1 replication (15, 27, 37, 38, 47). A mechanism that has been proposed to explain the infection of memory CD4-expressing T cells states that HIV-1-infected resting memory CD4\(^+\) T lymphocytes derive actually from activated naive CD4\(^+\) T cells that become infected and then revert to a resting G\(_0\) state (7). However, it has also been postulated that the predominantly by which memory CD4\(^+\) T lymphocytes in a quiescent state get
infected is through direct infection. The present study is thus in full support of this hypothesis. Indeed, we provide evidence that memory CD4+ T cells are specifically targeted by ICAM-1-bearing HIV-1 particles when infection is carried out in a heterogeneous cell population made of both naive and memory subsets. We demonstrate that the presence of numerous LFA-1 molecules organized in clusters is a key element for a more efficient attachment of HIV-1 particles carrying host ICAM-1 onto memory CD4+ T cells, an event that is also associated with an augmentation of virus entry and a higher level of integrated viral DNA.

Our findings are perfectly in line with a study that has estimated the number of resting naive and memory CD4+ T cells that are infected under in vivo situations in HIV-1-seropositive subjects. In fact, Ostrowski and colleagues have reported that memory CD4+ T cells had a median of fourfold more replication-competent virus and 16-fold more integrated provirus than naive CD4+ T cells in a cohort of 11 patients (39). Another study has also demonstrated that memory T cells from HIV-1-infected patients contain an average of 10 times more copies of HIV-1 DNA than naive T cells (10). In the present study, we found that memory CD4+ T cells carry 10 to 22 times more integrated viral DNA upon infection with ICAM-1-bearing virions than resting naive CD4+ T cells or than these two cellular subsets infected with isogenic viruses lacking ICAM-1. Furthermore, our data reveal that virus production is around 3.4 times greater in quiescent memory CD4+ T cells infected with ICAM-1-bearing HIV-1 particles compared to naive CD4+ T lymphocytes. Overall, our results indicate that memory CD4+ T cells are more permissive to productive infection with HIV-1 particles that bear host-encoded ICAM-1. The physiological relevance of this observation should not be underestimated considering that ICAM-1 has been reported to be acquired by all studied clinical and laboratory variants of HIV-1 independently of the tropism, which were amplified in primary human cells including histocultures of lymphoid tissue (2, 9, 12, 14, 23, 36).

Data from virus capture tests performed with NL4-3 particles that were produced in transiently transfected 293T cells and more natural cell targets (i.e., PBMCs and human lymphoid tissue cultured ex vivo) indicate that viruses from 293T cells are more efficiently captured than virions amplified in primary human cells (data not shown). This might suggest that virions produced by 293T cells incorporate larger amounts of host ICAM-1 than viruses that are shed by primary human cells. Although such findings question the significance of the current experiments, it is important to emphasize that previous work carried out with progeny virus produced in PBMCs provided evidence of the dominant role played by interactions between virus-anchored ICAM-1 and cell surface LFA-1 in HIV-1 attachment, entry, and infectivity through the use of blocking agents such as neutralizing antibodies (i.e., MEM25, MEM30, and RR1/1.1.1) and lovastatin (3, 9, 24, 55). Moreover, it was recently demonstrated that the lateral mobility and clustering of LFA-1 are crucial in the early events of HIV-1 life cycle for viruses produced in both 293T cells and PBMCs (56).

FIG. 5. Attachment and entry of viruses either lacking or bearing host-derived ICAM-1 in quiescent and activated CD4+ T-cell subsets. (A) Naive and memory quiescent or activated CD4+ T cells (i.e., treated with PHA and IL-2 for 24 h) were either untreated or treated withLovastatin (50 μM) or pravastatin (50 μM) for 30 min at 37°C before an incubation for 6 h with HIV-1 particles either devoid or bearing ICAM-1. Cells were next washed, and naive and memory CD4+ T cells were separated as described in Materials and Methods. Cells were maintained in culture in the presence of PHA and IL-2 for an additional 72 h, and supernatants were harvested to estimate virus production. Experiments were performed in triplicate, and standard deviations are indicated. Results shown are representative of three different experiments.

FIG. 6. HIV-1 infection of a heterogeneous CD4+ T-cell population. Unseparated resting CD4+ T cells were either left untreated or treated withLovastatin (50 μM) or pravastatin (50 μM) for 30 min at 37°C before an incubation for 6 h with HIV-1 particles either devoid or bearing ICAM-1. Cells were washed, and naive and memory CD4+ T cells were separated as described in Materials and Methods. Cells were maintained in culture in the presence of PHA and IL-2 for an additional 72 h, and supernatants were harvested to estimate virus production. Experiments were performed in triplicate, and standard deviations are indicated. Results shown are representative of three different experiments.
could relate to the use of a different anti-LFA-1 antibody that is specific for CD18 (β2-chain of LFA-1, clone 685A5) while we have used a blocking anti-CD11a antibody (α-chain of LFA-1). Despite this dissimilarity, both studies demonstrate that the inability of ICAM-1-bearing virions to productively infect naive CD4+ T cells can be reversed by the activation of this cell subset with PHA/IL-2, a process which is known to induce the expression of LFA-1 molecules and their redistribution in clusters.

It has been shown that reorganization of LFA-1 molecules in clusters represents the major mechanism regulating the association between ICAM-1 and LFA-1 (18, 31, 59–61). It is well known that LFA-1 is maintained in a low avidity state in naive CD4+ T cells by the tethering of the integrin tail to the actin cytoskeleton (45, 48). Cellular activation leads to the release of LFA-1 molecules from its cytoskeleton constraint, favoring lateral diffusion and the formation of LFA-1 clusters (18, 28, 52, 64). As memory CD4+ T cells originate from activated naive CD4+ T cells, where LFA-1 molecules are distributed in clusters, it was not surprising to discover LFA-1 clusters on resting memory CD4+ T cells. The presence of these clusters may be independent of cellular activation, since resting memory CD4+ T cells do not express activation markers such as CD25 and CD69 (data not shown). To the best of our knowledge, it represents the first demonstration that LFA-1 molecules are patched on the surface of memory CD4+ T cells.

Given that biochemical events can be transduced in target cells by HIV-1-anchored host proteins (8), it can be postulated that attachment of ICAM-1-bearing virions to the surface of LFA-1-expressing cells might trigger outside-in signaling. This could, in turn, as suggested previously (55), favor a firmer docking of the viral entity onto the target cell surface due to ICAM-1 activation and a possible mobilization of LFA-1 to the lipid raft and thus move it closer to CD4. Moreover, it can be proposed that LFA-1-mediated signal transduction events due to engagement with virus-anchored host ICAM-1 can lead to a remodeling of the cytoskeleton, a process that might lead to a destabilization of the plasma membrane and a higher probability to interact with the appropriate chemokine coreceptor CXCR4. The previous demonstration that the F-actin cytoskeleton is remodeled by ICAM-1-dependent signaling through LFA-1 fully supports this scenario (40).

Although it cannot be excluded that the naive cell subpopulation is totally free of cells expressing a nonnaive phenotype, it should be noted that nonnaive CD45RA CD4+ T cells represent a very small fraction of effector CD4+ T lymphocytes which usually express the CD45RO isoform. To confirm that our purified CD45RA population was truly naive, we performed infectivity experiments with R5-tropic virions based on the notion that the chemokine receptor CCR5 is not expressed on naive CD4+ T cells but is expressed by the effector subset (44). Virus replication was not detected in the CD45RA cell fraction (data not shown), thus confirming that purified CD45RA CD4+ T cells are virtually free of nonnaive CD45RA cells.

Beside phenotypic differences between naive and memory CD4+ T cells, these two distinct cellular subpopulations also exhibit differences with respect to their functional properties and metabolism. Indeed, memory T cells seem to be metabolically more active than naive T cells (53). As memory T cells contain higher levels of RNA and proteins than naive T cells, it has been suggested that the memory subset may be resting in the G_{1} rather than the G_{0} state (53). These properties probably also partly explain the greater susceptibility of these cells to HIV-1 infection. Conversely, naive and memory T cells react differently to cellular activation (4, 5). Several differences in the two T-cell subsets have been noted that could affect signaling pathways within these cells, which may explain different susceptibility to viral replication (20, 25, 33). Preferential HIV-1 replication in the memory cell subpopulation appears to be related to a greater stimulus-mediated activation of the nuclear factor of activated T cells in this cell type than what is seen in naive T cells (42). Nuclear factor of activated T cells has been demonstrated to act in synergy with the nuclear factor kappa B for promoting HIV-1 transcription in both T-cell lines and primary human T cells (1, 17, 29, 30). This is not surprising because HIV-1 gene expression is known to rely on a complex interplay between a series of cellular transcription factors and the viral encoded transactivator of transcription Tat. This might help to explain that, even though comparable amounts of ICAM-1-bearing HIV-1 particles entered within activated naive and memory CD4+ T cells, we found that virus production is superior in cells displaying the memory phenotype.

In conclusion, our findings propose a possible mechanistic framework explaining the higher susceptibility of memory CD4+ T cells to HIV-1 infection. The role played by virus-associated host ICAM-1 and cell surface LFA-1 under a cluster formation might be considered a determining factor in the initial events of HIV-1 life cycle since we demonstrate that it can influence the permissiveness of quiescent memory CD4+ T cells to a productive virus infection. The propensity of HIV-1 to preferentially target memory CD4+ T cells that contain LFA-1 clusters might simply reflect a biological adaptation the virus has evolved to establish a sanctuary of long-lived cells that carry integrated viral DNA.

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