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Entry and Transcription as Key Determinants of Differences in CD4 T-Cell Permissiveness to Human Immunodeficiency Virus Type 1 Infection†

Angela Ciuffi,[1] Gabriela Bleiber,[1] Miguel Muñoz,[1] Raquel Martinez,[1] Corinne Loeuillet,[1] Manuela Rehr,[2] Marek Fischer,[3] Huldrych F. Günthard,[3] Annette Oxenius,[2] Pascal Meylan,[1] Sebastian Bonhoeffer,[4] Didier Trono,[5] and Amalio Telenti[1]*

Institute of Microbiology, University of Lausanne, Lausanne,[1] Institute for Microbiology[2] and Ecology and Evolution,[4] ETH Zurich, and Division of Infectious Diseases and Hospital Epidemiology, University Hospital Zurich,[3] Zurich, and Department of Genetics and Microbiology, University of Geneva, Geneva,[5] Switzerland

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Isolated primary human cells from different donors vary in their permissiveness—the ability of cells to be infected and sustain the replication of human immunodeficiency virus type 1 (HIV-1). We used replicating HIV-1 and single-cycle lentivirus vectors in a population approach to identify polymorphic steps during viral replication. We found that phytohemagglutinin-stimulated $CD4^+$ $CD45RO^+$ $CD57^-$ T cells from healthy blood donors ($n = 128$) exhibited a 5.2-log-unit range in virus production. For 20 selected donors representing the spectrum of CD4 T-cell permissiveness, we could attribute up to 42% of the total variance in virus production to entry factors and 48% to postentry steps. Efficacy at key intracellular steps of the replicative cycle (reverse transcription, integration, transcription and splicing, translation, and budding and release) varied from 0.71 to 1.45 log units among donors. However, interindividual differences in transcription efficiency alone accounted for 64 to 83% of the total variance in virus production that was attributable to postentry factors. While vesicular stomatitis virus G protein-mediated fusion was more efficacious than CCR5/CD4 entry, the latter resulted in greater transcriptional activity per proviral copy. The phenotype of provirus transcription was stable over time, indicating that it represents a genetic trait.

The genetic makeup of individuals plays a role in susceptibility to human immunodeficiency virus type 1 (HIV-1) infection and progression of disease. Some of the observed variations have been attributed to immunogenetic diversity (major histocompatibility complex homozygosity and specific HLA types) and polymorphisms in chemokines, chemokine receptors, and cytokine genes (those for CCR5, CCR2, CX3CR1, SDF1, MIP1α, RANTES, interleukin-10 [IL-10], and IL-4) (5, 35). Isolated primary human cells from different donors also vary in their permissiveness—the ability of cells to be infected and sustain the replication of HIV-1 (41). Spira and Ho infected peripheral blood mononuclear cells (PBMCs) from 10 healthy donors with a wide array of viral isolates and laboratory strains and reported a 40-fold range of differences in infection among individuals (31). Eisert et al. analyzed parameters influencing the susceptibility of cells of the monocyte/macrophage lineage isolated from 30 healthy donors (11). The proportion of infected cells ranged from 0.03 to 99%, and the reverse transcriptase activity ranged from undetectable to 5×10^6 cpm/ml over 90 min. Infection of macrophages derived from pairs of identical twins displays a high concordance in the kinetics of HIV-1 replication (6, 23), underscoring the role of genetic factors in determining individual cell susceptibility to infection.

The HIV-1 life cycle is characterized by numerous interactions with host cellular proteins (14, 25). While restriction at entry plays a key role in determining infection kinetics (4, 26), there is limited knowledge on whether host proteins involved in other steps of the viral life cycle contribute to interindividual susceptibility to HIV-1 infection. Certain proteins are necessary for infection and for sustaining viral replication, while others represent antiviral factors. Some cellular antiviral factors can be selectively suppressed by viral proteins, as shown by the interaction between APOBEC3G, a cytidine deaminase, and Vif (15, 18–20, 30). An early interplay between incoming retroviral preintegration complexes and the nuclear proteins integrase interactor 1 and promyelocytic leukemia protein creates an antiviral state that interferes with the immediate-early steps of HIV-1 infection (37). ZAP, a zinc finger protein, inhibits the production of retroviral RNA (12). A number of antiretroviral factors target the capsid protein, imposing a postentry block (16, 32). This growing list of specific antiretroviral factors adds to current knowledge on antiviral innate defense mechanisms implicating interferon responses mediated by double-stranded RNA-dependent protein kinase, the MX proteins, and RNase L-mediated degradation of viral RNAs (13). Genetic polymorphisms in antiviral genes or in host genes participating in the viral life cycle could thus result in differences in the levels of expression or in the functional potential of protein variants and thus lead to differences in permissiveness to HIV-1 infection.

To address these issues, we (i) defined the variability in the permissiveness of CD4 T cells in a human population, (ii) expanded T cells from individuals representing the spectrum of

* Corresponding author. Mailing address: Institute of Microbiology, 1011 Lausanne, Switzerland. Phone: 41 21 314 0550. Fax: 41 21 314 4095. E-mail: amalio.telenti@hospvd.ch.

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cellular susceptibility to HIV-1 infection, (iii) used assays to measure the progress of the virus through the different replication steps in the cells and the variability of each step in a population, and (iv) estimated the contributions of various intracellular blocks to the overall permissiveness of the cells.

MATERIALS AND METHODS

Cells. CD4 T cells from 128 healthy Caucasian blood donors were isolated with anti-CD4 magnetic beads (Miltenyi Biotech) and cultured in vitro in RPMI 1640 (Gibco-Invitrogen) supplemented with 20% fetal calf serum (FCS), human IL-2 (Roche) at 20 U/ml, and gentamicin at 50 μg/ml (R-20) following stimulation with phytohemagglutinin (PHA) at 2 μg/ml for 2 days. CD4 T cells were expanded in R-20 in the presence of irradiated PBMCs and PHA at 2 μg/ml. Immunotypic characterization of the collection included flow cytometric analysis of the expression of RANTES (R&D Systems), MIP1β (R&D Systems), tumor necrosis factor alpha (Becton Dickinson [BD]), gamma interferon (BD), IL-2 (BD), IL-4 (BD), IL-10 (BD), CCR5 (BD), HLA-DR (BD), CD25 (BD), CD57 (BD), and CD69 (BD). Cells were genotyped for the CCR5Δ32 deletion.

The collection was assessed for permissiveness to HIV-1 infection in vitro under standardized conditions with NL4-3BaL*env*, an R5 infectious clone generated by substituting the SalI-BamHI fragment of pNL4-3 (encompassing most of the *env* open reading frame) with the corresponding fragment of pBaL (21). CD4 T cells (10^6 cells) were infected with 1,000 pg of p24 antigen of NL4-3BaL*env* in a 1-ml final volume for 2 h at 37°C in 5% CO_2. Cells were washed and cultured in R-20 for 10 days. Virus-containing supernatant was harvested, and p24 antigen production was monitored by an enzyme-linked immunosorbent assay (ELISA) (Abbott). Temporal trends in permissiveness were investigated for three donors by isolating CD4 T cells at three time points over a 9-week period.

Vectors and viral production. pHIVΔEnv was generated by deleting a 1,206-bp KpnI-BsaBI fragment in the gp120 coding region of NL4-3BaL*env*. pHIV/RTminus was generated by introducing a D185E mutation in the reverse transcriptase active site. Viral production was carried out with 293T cells. For pseudotyping, cells were cotransfected by the calcium phosphate technique with pHIVΔEnv and with a plasmid carrying the vesicular stomatitis virus (VSV) G protein or the HIV-1 R5-tropic JRFL envelope. The lentivirus LvGFP was produced by cotransfecting the Gag-Pol construct (pCMV.ΔR8.92), the Rev expression plasmid (pRSVRev), the VSV G protein envelope construct (pMD.G), and the packaged construct (pWPT-GFP) expressing green fluorescent protein (GFP) (see www.tronolab.unige.ch for vector details).

Analysis of intracellular steps. CD4 T cells (600,000 cells) were transduced with 10,000 pg of p24 of HIVΔEnv/VSV or 100,000 pg of p24 of HIVΔEnv/R5 in the presence of Polybrene (Sigma, Buchs, Switzerland) at 2 μg/ml in R-20 by spinoculating cells for 3 h at 1,500 × *g* and 22°C (24). Cells were washed and harvested 12 h and 4 days posttransduction for the extraction of nucleic acids and for the assessment of cell-associated and extracellular p24. RNA was extracted by using an RNeasy kit (Qiagen) and reverse transcribed by using Expand reverse transcription-PCR (Roche) with random hexamers. DNA was extracted by using a QiaAmp DNA extraction kit (Qiagen). cDNA and DNA were used for Taqman quantitative PCR (Applied Biosystems) of total viral RNA (*nef* primers), unspliced RNA (*gag* primers), and 12-h and 4-day HIV-1 DNA (*gag* primers), normalized with the housekeeping genes and transcripts of β-actin and cyclophilin A. Multiply spliced RNAs (*tat*, *rev*, and *nef* primers) were quantified by a direct one-step reverse transcription-PCR protocol (11a) and normalized to β-actin mRNA. The primers and probes used are shown in Table S1 in the supplemental material.

8E5/LAV cells carrying a single defective proviral copy of HIV-1 were used as a standard to quantify HIV-1 integrated copies. Synthetic RNA and cDNA dilution series from one donor were used to establish standard curves for RNA. For experiments with LvGFP, 600,000 cells were transduced with increasing amounts of the LvGFP construct as described above. GFP expression was assessed by flow cytometry 3 and 6 days posttransduction.

Postentry restriction. To examine the possibility that expanded CD4 T cells present a postentry block due to a saturable restriction factor (16), we preexposed CD4 T cells (300,000 cells) to a high dose of defective HIV/RTminus prior to infection with NL4-3BaL*env*. CD4 T cells were exposed to HIV/RTminus (300,000 pg of p24) for 4 h, washed, and infected with 300 pg of p24 of NL4-3BaL*env* for 2 h. Supernatant was harvested 5 and 7 days postinfection and monitored by a p24 ELISA. Similarly, CD4 T cells were transduced with LvGFP after preexposure to HIV/RTminus, and GFP expression was assessed by flow cytometry.

Infectivity assay. Supernatant from day 7 replication kinetic assays was harvested and used in infectivity assays. Ghost-CCR5 cells (30,000 cells) (Central Facility for AIDS Reagents, NIRSC, MRC, London, United Kingdom) were infected by spinoculation with 2,000 pg of HIV-1 p24. At 24 h postinfection, Ghost-CCR5 cells were washed with phosphate-buffered saline, trypsinized, fixed, and analyzed for GFP expression by flow cytometry.

CC-chemokine secretion. Culture supernatants were harvested from stimulated CD4 T cells before and 10 days after HIV-1 infection. Supernatants were filtered (0.22-μm-pore-size filters) and heat inactivated (30 min at 60°C), and RANTES, MIP1α, and MIP1β levels were assessed by an ELISA (R&D Systems).

Apoptosis. CD4 T cells (500,000 cells), uninfected or HIV-1 infected for 5 days, were stained with annexin V-fluorescein isothiocyanate (BD) according to the manufacturer's instructions and analyzed by flow cytometry.

Statistical analysis and modeling. Multiple regression analysis was performed by using the statistics package R (www.r-project.org). All values were log transformed. For both viral constructs, HIVΔEnv/R5 and HIVΔEnv/VSV, we first regressed extracellular p24 against all predictors (i.e., cDNA, proviral DNA, viral mRNA, and cell-associated p24) simultaneously. The predictors with least significance then were removed by stepwise backward elimination as implemented by the function step in statistics package R. The best model was chosen based on the Akaike information criterion, which balances the quality of the fit against the number of predictors included in the model.

RESULTS

Interindividual differences in permissiveness to HIV-1 infection. After 7 days of infection, CD4 T cells displayed a 5.2-log-unit range in p24 antigen production, i.e., in permissiveness to HIV-1 infection (Fig. 1A). CD4 T cells from 20 individuals chosen to represent the extremes and the spectrum of permissiveness were expanded in vitro to 10^8 cells. Expanded CD4 T cells consisted of a >97% pure population of $CD45RO^+$ $CD57^-$ T lymphocytes—the cells most susceptible to HIV-1 (1). These purified populations also displayed a 5-log-unit range in susceptibility to HIV-1 infection (3.5 to 4.8 log units when homozygous carriers of the CCR5Δ32 allele were excluded) and, overall, presented the same permissiveness phenotype as the original native cells (Fig. 1B). Although there were differences among the various CD4 T-cell populations in growth rates (mean and standard error of the mean [SEM], 1.5-fold ± 0.2-fold after 3 days postinfection) or in apoptosis (mean and SEM, 7.6% ± 0.7% apoptotic cells 5 days postinfection), we did not observe significant contributions of these factors to the permissiveness phenotype (for growth rates: R^2, 0.16, and P, 0.11; for apoptosis: R^2, 0.02, and P, 0.45) (see Fig. S1 and S2 in the supplemental material).

The cells were characterized on the basis of the expression of cytokines and chemokines. There were differences in the frequencies of cells expressing RANTES (0.04% ± 0.007%), MIP1β (11.1% ± 2.2%), tumor necrosis factor alpha (20.7% ± 3.0%), gamma interferon (20.7% ± 2.4%), IL-2 (9.0% ± 0.78%), IL-4 (3.4% ± 0.32%), and IL-10 (0.24% ± 0.05%) (all reported as mean and SEM). The activation status of the cells was assessed by determining the frequencies of cells expressing HLA-DR (80.9% ± 2.6%), CD25 (4.4% ± 0.8%), and CD69 (96.2% ± 0.4%) (all reported as mean and SEM). For all of the above markers, there was no association between flow cytometry results and permissiveness phenotype (Fig. 2).

Flow cytometry of CCR5 expression on uninfected CD4 T cells demonstrated a 0.4- to 0.6-log-unit range in mean fluorescence intensity. Differences in CCR5 density correlated significantly with cell permissiveness (R^2, 0.42; P, <0.0001) (Fig. 3A). As CC-chemokine levels could compete with HIV-1 for

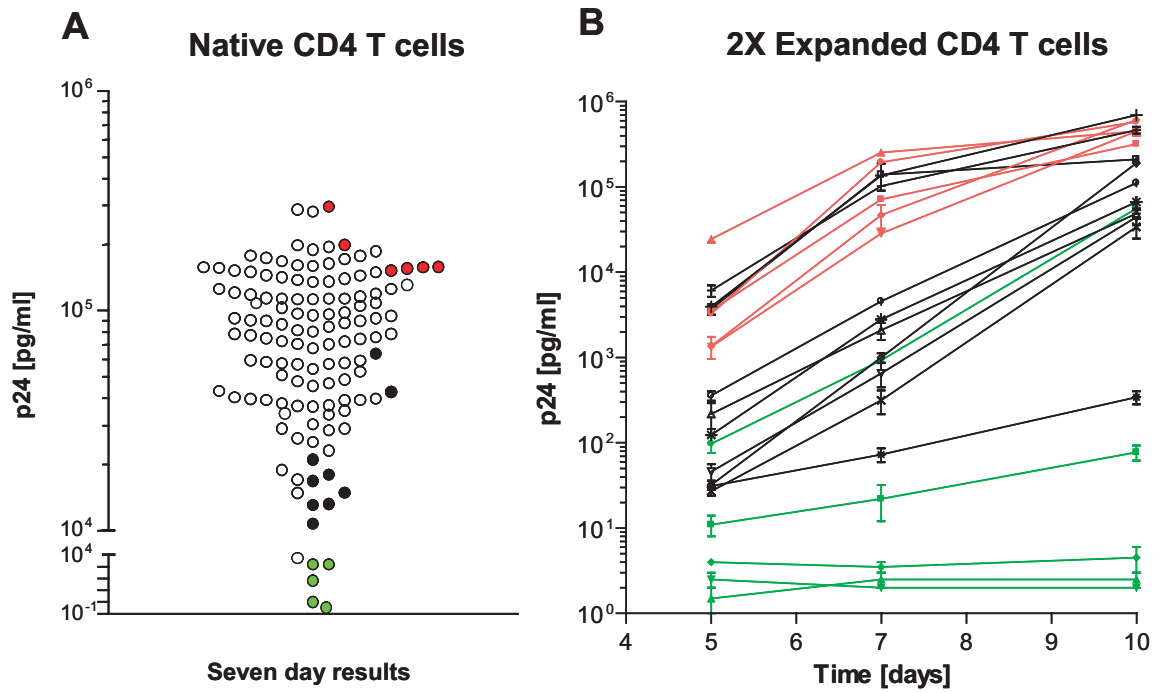

FIG. 1. Replication kinetics for HIV-1 in native or expanded CD4 T cells. (A) Cells isolated from 128 healthy blood donors were stimulated in vitro for 2 days with PHA and infected with 1,000 pg of p24 of R5-tropic NL4-3BaL*env*. Infection was monitored as the p24 antigen level in the supernatant. (B) CD4 T-cell populations ($n = 20$) representative of the spectrum of permissiveness to HIV-1 were expanded twice in vitro with irradiated PBMCs, PHA, and IL-2 and infected. Error bars indicate SEMs. Shown are hyperpermissive (red), normally permissive (black), and hypopermissive (green) cells.

binding to CCR5 and thus impair HIV-1 replication, we looked for differences in RANTES, MIP1α, and MIP1β levels in supernatants of CD4 T-cell cultures before or 10 days after HIV-1 infection. We observed a 0.9- to 1.5-log-unit range in CC-chemokine levels that did not correlate with differences in permissiveness (see Fig. S3 in the supplemental material). These data are consistent with prior reports (11) and with the observation that although CC-chemokines are generally viewed as inhibitory molecules, they block R5 viruses only at very high concentrations (8).

To identify differences in permissiveness that are not attributable to entry variability, we infected cells with viral constructs that differ only in the entry pathway. For this, HIVΔEnv was pseudotyped with the R5-tropic JRFL envelope or with the VSV G protein envelope and transduced into selected CD4 T-cell populations. A comparison of amounts of p24 antigen released from a single round of infection for the two viruses revealed that shared intracellular steps explained 48% of the total variance in virus p24 antigen production (R^2, 0.48; P, 0.0014) (Fig. 3B).

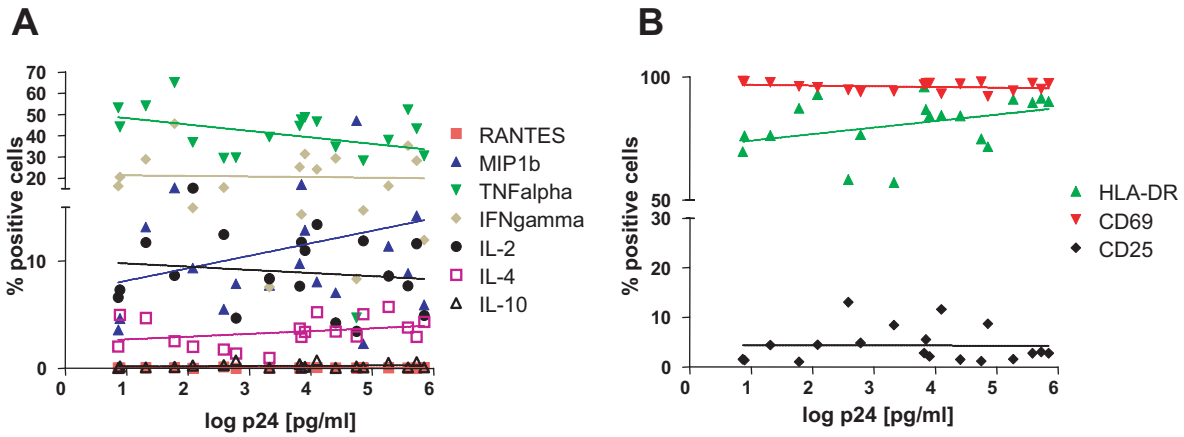

FIG. 2. Immunological characterization of expanded CD4 T-cell pools. The expression of selected cytokines and chemokines (A) and surface activation markers (B) was analyzed by flow cytometry. CD4 T-lymphocyte pools represented a >97% pure population of $CD45RO^+$ $CD57^-$ T cells. All correlations (R^2) were <0.15, and P values were >0.1. TNF, tumor necrosis factor; IFN, interferon.

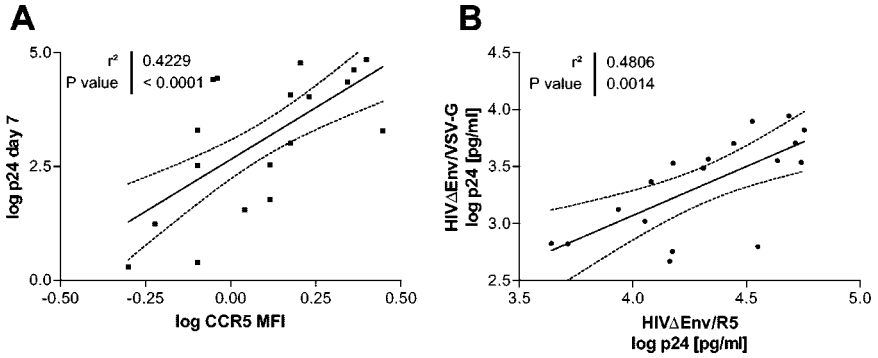

FIG. 3. Contributions of entry and nonentry host factors to cell permissiveness. (A) CCR5 receptor density, expressed as mean fluorescence intensity (MFI), correlated with p24 antigen production measured 7 days after infection with R5-tropic NL4-3Ba*Lenv* (R^2, 0.42; P, <0.0001). Similar estimates were obtained for percentages of $CCR5^+$ cells. (B) Assessment of entry-independent factors influencing cell permissiveness. Viral vectors differing only in the mechanism of entry—HIVΔEnv pseudotyped with the R5-tropic JRFL envelope (10^5 pg of p24) and with the VSV G protein envelope (10^4 pg of p24)—were used to infect 6×10^5 CD4 T cells. The correlation (R^2, 0.48; P, <0.0014) represents the shared contributions of intracellular steps to the cell permissiveness phenotype, measured as the amount of p24 in the supernatant 4 days posttransduction. Not shown in this analysis are values for cells from two donors homozygous for CCR5Δ32. Each point represents the mean of triplicate values.

Variations in viral life cycle steps. We quantified viral nucleic acids and proteins that accumulate at different times during the viral life cycle in order to assess efficiency at each infection step in cells from different individuals. We found that all postentry steps—reverse transcription (cDNA copies at 12 h postinfection), integration (proviral copies at 4 days), transcription (viral mRNA copies at 4 days), translation (cell-associated p24 antigen at 4 days), and budding and release (extracellular p24 and the ratio of extracellular p24 to cell-associated p24)—were variable. Interindividual differences ranged from 0.77 to 0.80 log units for the reverse transcriptase step up to 1.45 log units for the number of integrated copies (Fig. 4A). HIV-1 integration events were estimated by *gag* PCR (total HIV-1 DNA) 4 days postinfection, as it has been shown that integrated DNA accounts for all HIV-1 DNA detected by 72 h postinfection (2, 3, 33, 34).

VSV G protein-mediated fusion was more efficacious than R5-tropic vector transduction (Fig. 4A, reverse transcription and integration), despite the use of a 1-log-unit-smaller inoculum. However, when results were evaluated per proviral DNA copy, we observed greater transcriptional activity after CD4/CCR5 entry than after VSV G protein-mediated fusion (Fig. 4B), consistent with a role for envelope-mediated signaling in determining the activation and transcriptional status of the cells (8).

Variations in reverse transcription and integration steps were explained to a large extent by random effects, as indicated by the low concordance of results for the two viruses (Fig. 5A1,

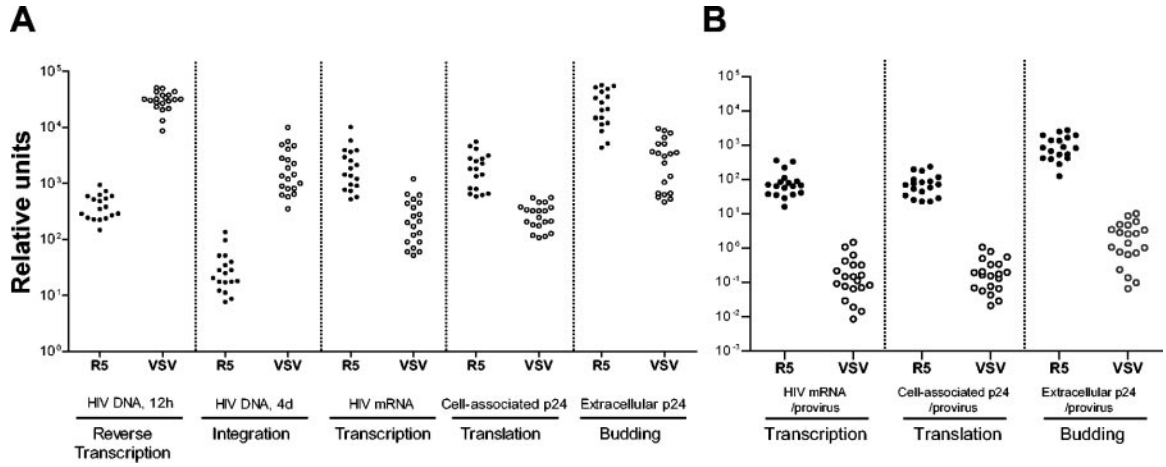

FIG. 4. Variations in intracellular steps. (A) Single-cycle experiments with HIVΔEnv pseudotyped with the R5-tropic JRFL envelope or with the VSV G protein envelope. Expanded CD4 T cells (6×10^5 cells) were infected with 10^5 pg of p24 of HIVΔEnv/R5 or 10^4 pg of p24 of HIVΔEnv/VSV. DNA samples were taken at 12 h and 4 days (4d) postinfection, and HIV-1 *gag* copies were normalized to the cyclophilin A copy number to quantify the efficiencies of reverse transcription and integration per cell. RNA samples were taken at 4 days postinfection, reverse transcribed with random hexamers, and assessed for HIV-1 mRNA copies normalized to β-actin mRNA. Cell-associated p24 and extracellular p24 were assessed at 4 days postinfection by an ELISA. (B) Reporting postintegration events relative to the number of proviral copies underscored the higher efficiency of transcription after CD4/CCR5 entry than after VSV G protein-mediated fusion. Not shown for R5 values are the results for two donors homozygous for CCR5Δ32. Each point represents the mean of triplicate values for one donor.

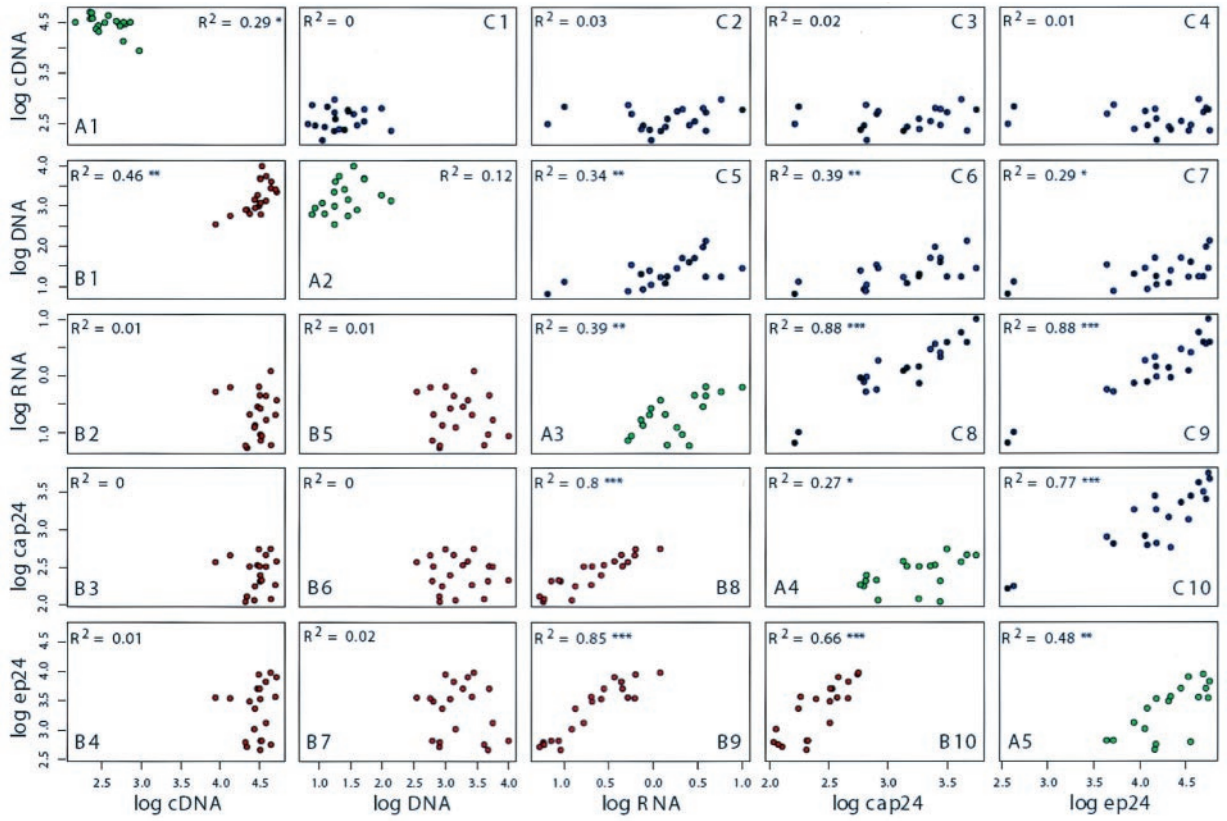

FIG. 5. Contributions of intracellular steps of the viral life cycle to cell permissiveness. Shown are pairwise correlation plots of measurements of 12-h viral cDNA (cDNA), proviral DNA (DNA), viral mRNA (RNA), cell-associated p24 (cap24), and extracellular p24 (ep24). The plots below and above the diagonal correspond to HIVΔEnv/VSV (red dots; B1 to B10) and HIVΔEnv/R5 (blue dots; C1 to C10), respectively. The diagonal plots (green dots; A1 to A5) represent the concordance between the viral constructs, but with the results for two donors homozygous for CCR5Δ32 excluded. Each point represents the mean of triplicate values for each donor. The correlations are displayed in the plots. Single, double, and triple asterisks indicate P values of <0.05, <0.01, and <0.001, respectively.

reverse transcription R^2, 0.29; Fig. 5A2, integration R^2, 0.12) and the poor reproducibility for the same vector over time (data not shown). These results were observed despite the optimal intratest reproducibility of triplicate values, with a test variation coefficient of less than 1%. In contrast, transcription, translation, and budding exhibited a higher concordance of results for the two viruses (Fig. 5A3 to A5, transcription R^2, 0.39; translation R^2, 0.27; budding R^2, 0.48) and good reproducibility for the same vector over time (data not shown).

Contributions of intracellular steps to the permissiveness phenotype. Variations in given intracellular steps of the viral life cycle were assessed by pairwise correlation plots of cDNA, proviral DNA, viral RNA, cell-associated p24, and extracellular p24 for both HIVΔEnv/R5 and HIVΔEnv/VSV (Fig. 5). For viruses pseudotyped with VSV G protein, variations in preintegration and integration steps correlated poorly with late-phase events or with interindividual differences in permissiveness (Fig. 5B1 to B7). This result was confirmed by using LvGFP to transduce CD4 T cells, where the vector proviral copy number was poorly correlated with the levels of GFP expression (R^2, 0.07; P, 0.095) (see Fig. S4 in the supplemental material). There was a better correlation between the proviral copy number and later steps for viruses with the R5-tropic envelope, driven in part by results from two CCR5Δ32 individuals (Fig. 5C5 to C7) and consistent with a gate effect of CCR5 entry (4). In contrast, variations in transcription, translation, and budding between individuals were highly correlated with the outcome of the infection, both for R5-tropic viruses (Fig. 5C8 to C10) and for viruses with a VSV G protein envelope (Fig. 5B8 to B10).

The HIV-1 transcription phenotype of each CD4 T-cell pool was common to HIV-1 vectors (transcribed from the long terminal repeat promoter) and to the LvGFP construct (transcribed from a cytomegalovirus promoter); the correlation (R^2) for HIVΔEnv/VSV mRNA and LvGFP expression was 0.49 (P, <0.0001) (see Fig. S4 in the supplemental material). β-Actin mRNA levels were lower in cells expressing higher levels of viral transcripts (for HIVΔEnv/VSV mRNA: R^2, 0.16, and P, 0.08). Analysis of unspliced and multiply spliced viral transcripts demonstrated an excellent correlation between different RNA species (R^2, 0.92; P, <0.0001), indicating the absence of significant polymorphisms in the host splicing machinery.

We examined the possibility that expanded CD4 T cells present a postentry block due to a saturable restriction factor. For this, we preexposed CD4 T cells to a high dose of defective HIV/RTminus prior to infection with NL4-3Ba*Lenv*. Consistent with previous reports that human cells do not restrict HIV-1 in a saturable fashion (16), we observed no inhibition (i.e., overcoming the postentry block generated by an inhibitory factor), as the levels of HIV-1 replication were compara-

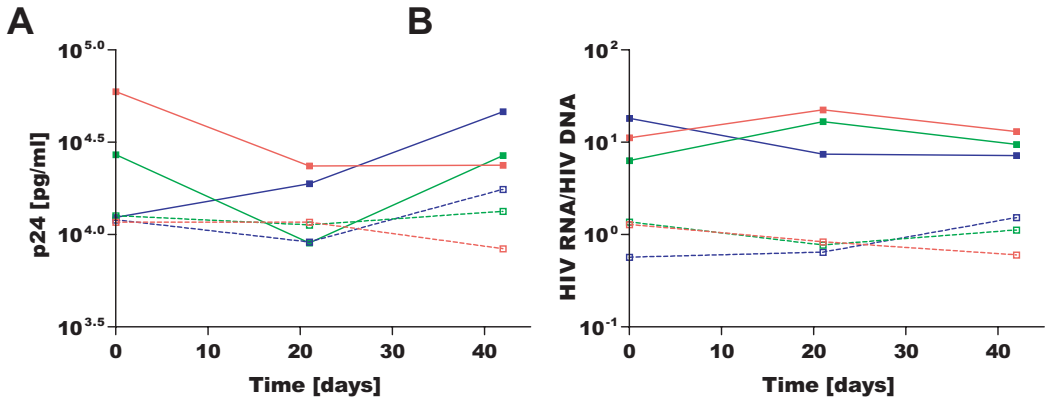

FIG. 6. Temporal trends in permissiveness. CD4 T cells were isolated from three donors (1, blue; 2, green; 3, red) at three different times (days 0, 21, and 42). Cells were transduced with HIVΔEnv/VSV (broken lines; 10^4 pg of p24) and HIVΔEnv/R5 (solid lines; 10^5 pg of p24) to infect 6×10^5 CD4 T cells. At 4 days postinfection, p24 antigen was measured in the supernatant (A), and real-time PCR was used to determine transcription per integrated provirus (B).

ble in cells preexposed and in cells not preexposed to the reverse transcriptase-defective virus (see Fig. S5 in the supplemental material).

Infectiousness of viral progeny. Differences in the activities of cellular antiviral factors or interindividual differences in particle maturation could have resulted in modifications of the ability of viral progeny to infect a reporter cell line. CD4 T-cell populations were infected with NL4-3BaL*env*, and the virus-containing supernatant was harvested 7 days postinfection, standardized for p24 antigen content, and used to infect Ghost-CCR5 cells. This procedure was possible only for 11 CD4 T-cell pools, the other cells not providing sufficient levels of virus in the supernatant to allow for testing under standardized conditions. A maximum twofold difference in infectiousness was observed and was correlated poorly with the overall cell permissiveness phenotype (R^2, 0.09; P, 0.046). However, the limited number of donors contributing to this analysis precludes a definitive conclusion regarding a role for particle infectiousness in differences in replication kinetics.

Modeling variations in the viral life cycle. Multiple regression of extracellular p24 against cDNA, proviral DNA, viral mRNA, and cell-associated p24 indicates that over 90% of the variance in p24 production is explained by the inclusion of these four factors. However, viral mRNA contributes 0.64 and 0.83 to the R^2 values for HIVΔEnv/R5 and HIVΔEnv/VSV, respectively, indicating that it is the most important single explanatory factor in the variance of extracellular p24. Model selection on the basis of the Akaike information criterion confirms that extracellular p24 resulting from infection by HIVΔEnv/R5 is best explained by the inclusion of only viral cDNA and mRNA as explanatory variables. The regression coefficient for $\log_{10}$ viral mRNA is 1.13 (P, $<10^{-11}$), and that for $\log_{10}$ viral cDNA is -0.71 (P, 0.005). Model selection for extracellular p24 produced by HIVΔEnv/VSV indicates that, in addition to viral mRNA and cDNA, proviral DNA is a significant explanatory factor. The regression coefficient for $\log_{10}$ viral mRNA is 1.03 (P, $<10^{-9}$), that for viral cDNA is 0.87 (P, 0.002), and that for proviral DNA is -0.31 (P, 0.012). Note that although the corresponding plots in Fig. 5C10 and B10 show a correlation between cell-associated p24 and extracellular p24 for both viral constructs, cell-associated p24 was nevertheless eliminated during model selection, since its inclusion did not significantly improve the model, a reflection of its strong dependence on viral mRNA levels.

Temporal trends in permissiveness. To assess whether permissiveness and transcription would represent stable traits of the individual, three healthy individuals donated cells at 3-week intervals for a total of three time points. Primary purified CD4 T cells were transduced with HIVΔEnv/R5 and HIVΔEnv/VSV. Interday variations in p24 antigen release were very constant for viruses pseudotyped with the amphotropic VSV G protein envelope, with maximal observed differences of 0.07 to 0.29 log pg of p24/ml (Fig. 6A). Transcription per proviral copy remained markedly stable for all three donors over time and for both transduced clones (Fig. 6B). In contrast, transduction with viruses pseudotyped with the R5-tropic JRFL envelope was less reproducible over time, with maximal observed differences of 0.40 to 0.57 log pg of p24/ml for the various donors (Fig. 6A). As in experiments with expanded CD4 T cells, we observed greater transcriptional activity after CD4/CCR5 entry than after VSV G protein-mediated fusion.

DISCUSSION

We sought to create a cellular system to measure interindividual differences in permissiveness to HIV-1 infection by using purified and in vitro-expanded CD4 T cells from healthy HIV-1-negative blood donors. This system allowed us to concentrate on the investigation of (intra)cellular factors modifying HIV-1 infection, thus removing confounding by immunological and immunogenetic factors. Cells were infected under highly standardized conditions with a laboratory clone to eliminate the variability associated with viral strain diversity (36). Thereafter, we used a number of techniques and viral vectors to assess the contributions of selected steps in the viral life cycle to the overall permissiveness phenotype.

This work identified a 5-log-unit range in p24 antigen production by CD4 T cells from healthy blood donors (3.5 to 4.8 log units when homozygous carriers of the CCR5Δ32 allele were excluded). Overall, 50% of the observed interindividual variations in CD4 T-cell permissiveness were derived from

entry-independent host factors. Analysis suggested that 64 to 83% of the variance in viral production was due to interindividual differences in provirus transcription. Results were derived from polyclonal infections, where transcription represents the outcome of several thousands of independent integration events, thus ruling out a positional bias (28).

Analysis of the transcriptome after infection with HIV-1, exposure of the cell to the viral envelope, or the expression of Nef has identified the up-regulation of a significant number of transcription activation genes (7, 8, 38, 39), including the nuclear factor of activated T cells (NFAT). The expression of NFAT is enhanced by PHA stimulation, and NFAT is sufficient as a cellular factor to induce a highly permissive state for HIV-1 replication (17). Given the number of transcription factors participating in the cellular response to HIV-1, we speculate that there is opportunity for the expression of genetic polymorphisms in one or more of the implicated genes. Despite the more efficient transduction for viruses pseudotyped with VSV G protein, which delivers HIV-1 into the endocytic compartment, CD4/CCR5 entry and envelope-mediated signaling (8) resulted in higher levels of transcription per proviral copy. As the viral clones differed solely in pseudotype, we can only conclude that the mode of entry determines the transcriptional status of the cell. Disappointing levels of infection by the R4 clone NL4-3 after the CD4 T cells were expanded, mainly because of down-regulation of the cell surface CXCR4 receptor, precluded a comparative analysis of CXCR4 restriction.

The experimental approach is challenging because of the complexity of using primary cells in a multitude of independent analyses. Maintaining cell viability and subjecting the cells to two cycles of expansion can lead to oligoclonal selection of CD4 T-cell subpopulations with heterogeneity in their permissiveness phenotypes. Although Mikovits et al. did not report significant differences between Th1 and Th2 CD4 cell clones with respect to permissiveness to HIV-1 (22), subpopulations of cells with reduced susceptibility to infection have been described in other settings (40). We used CD4 T-lymphocyte populations comprising $CD45RO^{+}$ $CD57^{-}$ T cells—the cells most susceptible to HIV-1 (1). Extensive analyses of intracellular and surface markers did not reveal profound differences among cells from the various donors, and the levels of cell activation markers in response to PHA stimulation did not correlate with differences in the permissiveness of the various CD4 T-cell pools. We are now establishing immortalized populations of cells from the various donors by using herpesvirus saimiri (27). Here, multiple clones can be established per individual, thus ensuring better control of intraindividual variability.

For most donors, the permissiveness phenotype of the primary cells was retained upon cellular expansion in vitro. In addition, we assessed the permissiveness and transcription phenotypes for cells obtained from the same individuals at separate (three) time points. Results were particularly reproducible when VSV-pseudotyped virions were used and when the number of viral transcripts per copy of proviral DNA was analyzed. These data would be in line with the results of studies of cells from twins, which underscore the stability and major contribution of genetic factors to cell permissiveness to HIV-1 (23).

We found that differences in the capacity of cells to proliferate, levels of apoptosis, and levels of expression of chemokines or cytokines did not correlate with the permissiveness phenotype. Additional characterization of the cells will be completed in the future by using different cell activation protocols, e.g., IL-7 stimulation (10). In addition, the degree of permissiveness may vary depending on the choice of viral strain for testing. Viruses that grow to a high level in cells from one donor do not necessarily do so in cells from another donor (6, 31). These data are fully consistent with the hypervariable nature of viral genomes, as polymorphisms in viral proteins may modify interactions with host factors.

As shown by the paradigm of the 32-nucleotide deletion in CCR5 (9), by the absence of APOBEC3G in a number of lymphoid and nonlymphoid cell lines (30), and by the relevance of protein variations in determining interspecies restriction of retroviruses (29, 32), identification of polymorphisms in relevant genes is key to unraveling the basis of HIV-1 pathogenesis. Thus, the observation that, after maximal cell activation, transcription polymorphism is just as important as entry variation should trigger a detailed analysis of genes participating in the activation, transcriptional status, and transcriptional machinery of cells.

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