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The Presence of Host-Derived HLA-DR1 on Human Immunodeficiency Virus Type 1 Increases Viral Infectivity

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Human immunodeficiency virus type 1 (HIV-1) incorporates several host cell components when budding out of the infected cell. One of the most abundant host-derived molecules acquired by HIV-1 is the HLA-DR determinant of the major histocompatibility complex class II (MHC-II) molecules. The fact that CD4 is the natural ligand of MHC-II prompted us to determine if such virally embedded cellular components can affect the biology of the virus. Herein, we report for the first time that the incorporation of cellular HLA-DR1 within HIV-1 enhances its infectivity. This observation was made possible with virions bearing or not bearing on their surfaces host-derived HLA-DR1 glycoproteins. Such virus stocks were prepared by a transient-expression system based on transfection of 293T cells with a recombinant luciferase-encoding HIV-1 molecular clone along with plasmids encoding the α and β chains of HLA-DR1. Cell-free virions recovered from transfected cells were shown to have efficiently incorporated host-derived HLA-DR1 glycoproteins. Infectivity was increased by a factor of 1.6 to 2.3 for virions bearing on their surfaces host-derived HLA-DR1. The observed enhancement of HIV-1 infectivity was independent of the virus stocks used and was seen in several T-lymphoid cell lines, in a premonocytoid cell line, and in primary peripheral blood mononuclear cells. Finally, we determined that the presence of virion-bound cellular HLA-DR1 is associated with faster kinetics of virus infection. Taken together, these results suggest that HLA-DR-1-bearing HIV-1 particles had a greater infectivity per picogram of viral p24 protein than HLA-DR1-free virions.

One common feature of retroviruses, as well as of many enveloped viruses, is the acquisition of host cell surface molecules during the budding process (2, 5, 17, 27, 43, 46). Human immunodeficiency virus types 1 and 2 (HIV-1 and -2) have also been shown to incorporate cell membrane-derived structures while budding out of the infected cell. It has been reported that cellular components, such as the HLA-DR determinant of major histocompatibility complex class II (MHC-II), the HLA class I α chain, β_2 microglobulin, LFA-1, ICAM-1, CD43, CD55, CD59, CD63, and CD71, are physically present on the virion surface (1, 8, 10, 20, 26, 29, 30, 49, 51, 57, 58).

Recently, host-derived molecules incorporated within HIV-1 were assigned functional roles in the viral life cycle. For example, virally associated glycosylphosphatidylinositol-linked CD55 and CD59 were demonstrated to protect virions from complement-mediated lysis (57). It has been suggested that virally acquired adhesion molecules may play a pivotal role in virus infection based on the marked synergy between plasma from HIV-1-infected individuals and an anti-LFA-1 monoclonal antibody in the neutralization of HIV-1 (28). Antiserum to HLA-DR was reported to result in an inhibition of HIV-1 infection, suggesting that this virally acquired host molecule is physically present on the surface of progeny virus (1). The biological function(s) of cellular HLA-DR glycoproteins acquired by HIV-1 has also been recently addressed, and it was found that bacterial superantigens can be efficiently presented by HLA-DR-bearing virions (55). Altogether, results from these studies indicate that host-derived molecules associated with budding HIV-1 are biologically active and putatively affect the pathogenesis of the disease.

The CD4 molecule is the primary cell surface receptor for HIV-1. This cell surface structure is implicated in the initial attachment step of the virus to the cell by interacting with the external viral glycoprotein gp120 (16, 38). $CD4^+$ T lymphocytes, which express high levels of surface CD4, represent the major cellular reservoir for HIV-1 in the peripheral blood, and their activation results in upregulation of virus replication (62). Cellular activation has also been shown to lead to an increase of surface expression of HLA-DR glycoproteins (18, 24, 37). Monocyte-derived macrophages, which are also $CD4^+$ and express HLA-DR in both constitutive and inducible manners (45, 54), are considered to be the most frequently identified hosts of HIV-1 in tissues of infected individuals (reviewed in reference 50). Consequently, the probability that newly formed virions will bear cellular HLA-DR is high. This postulate is supported by the observation that low-passage clinical isolates of HIV-1 grown on primary phytohemagglutinin (PHA)-stimulated peripheral blood mononuclear cells (PBMCs) carry on their surfaces host-derived HLA-DR (7). The physiological relevance of cellular HLA-DR bound to HIV-1 is further supported by previous studies indicating that HLA-DR is one of the most abundant host-derived molecules carried by HIV-1 (1, 7, 51) and by the finding that relative amounts of virally embedded host HLA-DR are influenced by both the viral strain and the producer cell line (7). Under physiological conditions, CD4 is the ligand of HLA-DR in the context of antigen presentation (6, 25). Assuming that the CD4 molecule on the target cell can bind to the virally embedded host HLA-DR, this additional interaction would result in a stronger association between the virus and its target cell. Thus, we have developed a transient-expression system that allowed us to produce virus stocks bearing or not bearing host-derived HLA-DR glycoproteins in order to study the role played by such virally incorpo-

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rated molecules during the viral infection process. In this report, we demonstrate that the presence of host-derived HLA-DR1 on virions results in an enhancement of HIV-1 infectivity.

MATERIALS AND METHODS

Cells. The human $CD4^+$ T-lymphoid cells CEM-T4 (23), Jurkat-*tat* (9), and Sup-T1 (60), as well as promonocytic U937 cells (63), were cultured in complete culture medium made up of RPMI 1640 medium (Gibco-BRL, Burlington, Ontario, Canada) supplemented with 10% fetal bovine serum (FBS; Hyclone Laboratories, Logan, Utah), glutamine (2 mM), penicillin G (100 U/ml), and streptomycin (100 mg/ml). These cell lines were obtained through the AIDS Research and Reference Reagent Program, Division of AIDS, National Institute of Allergy and Infectious Diseases, National Institutes of Health. The human embryonic kidney cell line 293T expressing the simian virus 40 large T antigen (53) was maintained in Dulbecco modified Eagle medium (DMEM; Gibco-BRL, Burlington, Ontario, Canada) supplemented with 10% FBS, glutamine (2 mM), penicillin G (100 U/ml), and streptomycin (100 mg/ml). 293T cells were kindly provided by Warner C. Greene (J. Gladstone Institutes, San Francisco, Calif.).

Primary PBMCs from a healthy donor were isolated by Ficoll-Hypaque density gradient centrifugation. Briefly, 10 ml of venous blood was layered on a Ficoll cushion and spun at 2,000 rpm for 30 min (Sorvall RT6000B; Du Pont Co., Wilmington, Del.). Mononuclear cells at the Ficoll interface were collected and washed twice in Hank's balanced salt solution. Thereafter, cells were resuspended at a density of 10^6 cells/ml in complete culture medium and cultured in the presence of 3 μg of PHA-P (Sigma, St. Louis, Mo.)/ml and 30 U of recombinant interleukin-2 per ml for 3 days at 37°C under a 5% CO_2 atmosphere. The following reagent was obtained through the AIDS Research and Reference Reagent Program: recombinant human interleukin-2 from Maurice Gately, Hoffman-La Roche Inc. (41).

Plasmids. The eukaryotic expression vectors pRSV.5 *neo* DRα, which encodes the HLA-DR α chain (47), and pRSV.3 DR1β, which encodes the HLA-DR1 β chain (32), were generous gifts from Rafick-Pierre Sékaly (Institut de Recherches Cliniques de Montréal, Montreal, Quebec, Canada). Both HLA-DR genes are controlled by the long terminal repeat of the Rous sarcoma virus. The proviral plasmid pHXB-Luc was originally derived from pHXB-2D, from which a part of the *nef* gene was deleted and replaced with the reporter *luciferase* gene (12). This infectious molecular clone of HIV-1 was kindly provided by David Baltimore (Massachusetts Institute of Technology, Cambridge, Mass.).

Preparation of virus stocks by transfection. All transfections were performed by following a modification of the calcium phosphate transfection protocol of Chen and Okayama (13). A typical transfection experiment was carried out with 10 μg of pHXB-Luc in the absence or presence of 1.25 μg of both pRSV.5 *neo* DRα and pRSV.3 DR1β. A plasmidic preparation(s) was mixed with 25 μl of 2.5 M $CaCl_2$, and the volume was completed to 250 μl with H_2O. This DNA preparation was then gently added to 250 μl of 2× HBS buffer (280 mM NaCl, 50 mM HEPES, 1.5 mM Na_2HPO_4; pH 7.05) and incubated at room temperature for 4 min, and the total solution was overlaid onto a semiconfluent monolayer of 293T cells that had been preinoculated 24 h before initiation of transfection in a six-well plate (Falcon; Becton Dickinson, Lincoln Park, N.J.) (5×10^5/well in 3 ml of DMEM supplemented with 10% FBS). At 16 h posttransfection, cells were washed twice with 3 ml of phosphate-buffered saline (PBS; pH 7.4) and incubated for 24 h in 3 ml of DMEM supplemented with 10% FBS. Virion-containing supernatants were filtered through a 0.45-μm-pore-size cellulose acetate membrane (Millipore, Bedford, Mass.), aliquoted in 500-μl fractions, and finally frozen at −85°C until needed. All virus stocks underwent one freeze-thaw cycle before initiation of infection studies. Virus stocks were normalized for virion content by a commercial assay for p24 (Organon Teknika, Durham, N.C.). The standardization of p24 contents was based on the observation that, in such virus preparations, the great majority of viral p24 is part of complete HIV-1 particles. Indeed, we determined that more than 95% of p24 is pelletable under ultracentrifugation conditions that are sufficient to sediment whole viruses (Heraeus Contifuge 28RS, 12,000 rpm for 90 min at 4°C). Amounts of p24 found in cell-free HXB-Luc preparations recovered following these transfection experiments ranged between 100 and 500 ng/ml. To ascertain that the amounts of intact virus particles used for infection experiments were indeed similar in HXB-Luc preparations with HLA-DR1 (HXB-Luc HLA-DR1/POS) and without HLA-DR1 (HXB-Luc HLA-DR1/NEG), we also monitored the reverse transcriptase activities of some virus stocks. Minimal differences for the reverse transcriptase activity/p24 ratios (in counts of reverse transcriptase activity per minute per 50 μl to the amount of p24 in nanograms per milliliter) were seen for HXB-Luc HLA-DR1/POS (ratio, 105.8) and HXB-Luc HLA-DR1/NEG (ratio, 93.6) preparations. Cotransfection of pHXB-Luc, pRSV.5 *neo* DRα, and pRSV.3 DR1β led to the production of the virus stocks called HXB-Luc HLA-DR1/POS, because such virions bear host-derived HLA-DR. Transfection of 293T cells with pHXB-Luc alone resulted in the production of the virus preparations named HXB-Luc HLA-DR1/NEG, since cellular HLA-DR glycoproteins are not found embedded within such virions.

Reverse transcriptase assay. Enzymatic activity was measured with 50 μl of virus preparations to which 10 μl of solution A (5 mM dithiothreitol [DTT], 50 mM KCl, 0.05% Triton X-100) and 40 μl of solution B [5 mM $MgCl_2$, 0.5 M EGTA, 0.04 mg of poly(rA)-oligo$(dT)_{12\text{-}18}$, 3 mCi of [^{3}H]TTP; 40 to 70 Ci/mmol] had been added. After incubation for 1 h at 37°C, samples were precipitated prior to filtration onto glass fiber filters by a cell harvester system (Tomtec). The filters were dried, and radioactivity was measured in a liquid scintillation counter (1205/1204 BS Beta-plate; Wallac Oy, Turku, Finland). The assays were performed in triplicate.

Antibodies. 31-90-25, an antibody recognizing the HIV-1 major core protein p24; L243, an antibody specific for a nonpolymorphic determinant of the HLA-DR αβ dimer (42); and 2.06, a monoclonal antibody directed against monomorphic HLA-DR (11), were used in our studies. These hybridomas were obtained from the American Type Culture Collection (Rockville, Md.). All monoclonal antibodies were isolated from hybridoma culture supernatants and purified by protein G-Sepharose affinity chromatography according to the manufacturer's (Gibco-BRL) instructions. 31-90-25 was biotinylated with NHS-LC-Biotin in accordance with the supplier's (Pierce, Rockford, Ill.) instructions.

Flow cytometric analyses of cell surface and internal antigens. The expression of HLA-DR1 on the surfaces of transiently transfected 293T cells was monitored with the anti-HLA-DR antibody L243. In brief, 10^6 transfected 293T cells were incubated for 30 min at 4°C with 100 μl of PBS containing 1 μg of L243, followed by two washes with 500 μl of PBS. Pelleted cells were resuspended in 100 μl of PBS containing 1 μg of fluorescein isothiocyanate-conjugated goat anti-mouse immunoglobulin G (IgG; Jackson ImmunoResearch Laboratories, Inc., West Grove, Pa.). Finally, cells were washed twice in PBS and resuspended in 300 μl of PBS containing 1% (wt/vol) paraformaldehyde before flow cytometry analysis (EPICS XL; Coulter Corporation, Miami, Fla.). Controls consisted of commercial isotype-matched murine monoclonal antibodies (Sigma). Intracellular viral p24 staining of transiently transfected 293T cells was performed with a commercial intracellular flow cytometry kit (Fix & Perm cell permeabilization kit; CALTAG Laboratories, South San Francisco, Calif.) in accordance with the supplier's instructions. This assay method was carried out with a combination of biotinylated 31-90-25 and R-phycoerythrin-conjugated streptavidin (Jackson ImmunoResearch Laboratories).

Immunodetection of virally acquired cellular HLA-DR1. The presence of host-derived HLA-DR1 glycoproteins on HIV-1 particles produced by transiently transfected 293T cells was monitored with antibody-coated magnetic beads as described previously (7). In brief, 12.5×10^6 magnetic beads (BioMag, Fc specific; PerSeptive Diagnostics, Inc., Cambridge, Mass.), previously coated with the anti-HLA-DR antibody 2.06, were incubated with similar amounts of HXB-Luc standardized in terms of the viral core p24 protein (2,500 pg of p24) in a final volume of 1 ml of binding medium (PBS plus 0.1% bovine serum albumin). This mixture was incubated for 1 h at 4°C on a rotating plate. The beads were washed five times in binding medium with a magnetic separation unit and were resuspended in 100 μl of binding medium. The amount of immunocaptured HIV-1 particles was assessed by measuring the viral p24 protein content found associated with immunomagnetic beads.

Virus infection and luciferase assay. In a 96-well flat-bottom tissue culture plate (Microtest III, Falcon; Becton Dickinson), 10^5 target cells were incubated at 37°C under a 5% CO_2 atmosphere with equal amounts of HXB-Luc HLA-DR1/POS or HXB-Luc HLA-DR1/NEG (1.5 ng of p24) in a final volume of 200 μl of complete culture medium. After an incubation period of 72 h, luciferase activity was monitored as described previously (3). In brief, 100 μl of cell-free supernatant was withdrawn from each well and 25 μl of 5× cell culture lysis buffer (125 mM Tris phosphate [pH 7.8], 10 mM DTT, 5% Triton X-100, 50% glycerol) was added before incubation at room temperature for 30 min. Thereafter, an aliquot of this cell lysate (20 μl) was mixed with 100 μl of luciferase assay buffer [20 mM Tricine, 1.07 mM $(MgCO_3)_4 \cdot Mg(OH)_2 \cdot 5H_2O$, 2.67 mM $MgSO_4$, 0.1 mM EDTA, 270 μM coenzyme A, 470 μM luciferin, 530 μM ATP, 33.3 mM DTT]. Emission of light produced by the reaction was measured with a liquid scintillation counter (model LS 6000TA; Beckman Instruments Canada, Inc., Mississauga, Ontario, Canada). Total photo events over 50 s were measured. In some experiments, the antiviral compound zidovudine (AZT) was added to cell culture media at a final concentration of 1 μM at 24 h postinfection.

Kinetics of HIV-1 infection were assessed as follows. Jurkat-*tat* cells were resuspended at a density of 10^6 cells/ml in complete culture medium and were inoculated with similar amounts of HXB-Luc HLA-DR1/POS or HXB-Luc HLA-DR1/NEG (1.5 ng of p24/10^5 cells). Viruses and target cells were incubated in a water bath at 37°C, and individual aliquots (100 μl) were taken at specific time points (0, 5, 10, 15, 30, 60, 120, 180, and 240 min). These aliquots were immediately transferred to wells of a 96-well flat-bottom tissue culture plate (Microtest III) containing 100 μl of complete culture medium supplemented with 20 μg of plasma-pooled purified human IgG from HIV-1-infected subjects. This step is required to stop as rapidly as possible the process of virus infection. The microtiter plate was next incubated at 37°C for 72 h, and luciferase activity was monitored as described above.

Statistical analysis. Results shown are expressed as means ± standard deviations of triplicate samples. Statistical analysis of the differences between groups was first performed by analysis of variance. If P values were less than 0.05, group comparisons were done by the Fisher least-significance difference post hoc test. A P value of less than 0.05 was considered significant. Calculations were made with Statview software.

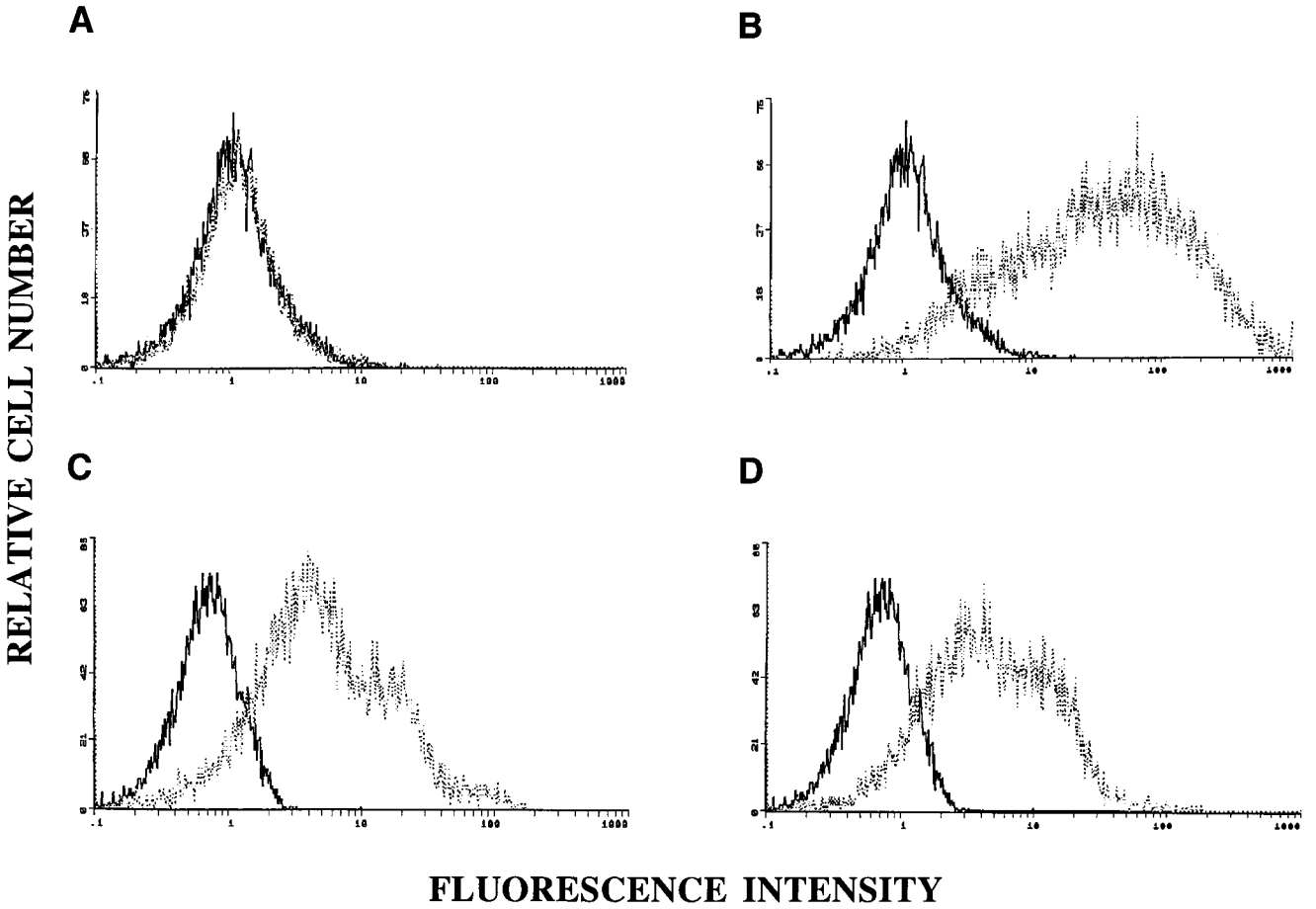

FIG. 1. Flow cytometry analysis of 293T cells transfected with pHXB-Luc and HLA-DR1-encoding plasmids. 293T cells were transfected with pHXB-Luc (A and C) or were cotransfected with pHXB-Luc, pRSV.5 *neo* DRα, and pRSV.3 DR1β (B and D). Surface HLA-DR was detected by staining cells with L243 (A and B), while measurement of intracellular viral p24 protein was achieved with biotinylated 31-90-25 (C and D). Cells were next incubated in the presence of fluorescein isothiocyanate-conjugated goat anti-mouse (A and B) or R-phycoerythrin-conjugated streptavidin (C and D) before flow cytometry analysis. Solid lines, untransfected cells; dotted lines, transfected cells.

RESULTS

Production of HXB-Luc virions bearing or not bearing host-derived HLA-DR1 glycoproteins. To verify whether the incorporation of host-derived HLA-DR can modulate the biological properties of HIV-1, we developed a transient-expression system that permitted the production of replication-competent virus particles carrying or not carrying on their surfaces host cell membrane HLA-DR. In this assay, progeny virus devoid of host HLA-DR was produced following transfection with an infectious HIV-1 molecular clone, while HLA-DR-bearing virions were generated following cotransfection with the same proviral HIV-1 clone along with plasmids encoding the HLA-DR α and HLA-DR1 β chains. Virus preparations from such transfected cells enabled us to determine if the intrinsic properties of HIV-1 are influenced by the acquisition of host-derived HLA-DR glycoproteins. To quantitatively monitor any changes in the early steps of the HIV-1 replicative cycle, we took advantage of the molecular clone pHXB-Luc, from which the *nef* gene has been partially deleted and replaced with the *luciferase*-encoding reporter gene. Upon transfection in a productive cell line, this molecular clone led to the production of fully infectious viruses. Furthermore, these viruses can be used to infect CD4-expressing cells and the extent of infection can be quantitatively analyzed by measuring luciferase activity.

Recombinant luciferase-encoding HIV-1 particles (HXB-Luc), bearing or not bearing host cell-derived HLA-DR, were produced by calcium phosphate transfection of the human embryonic kidney cell line 293T. These cells have no detectable surface expression of any MHC-II isotype (HLA-DR, -DP, and -DQ) (data not shown). Expression of HLA-DR on the surface of 293T cells was achieved by transfecting pRSV.5 *neo* DRα and pRSV.3 DR1β. The use of these two mammalian expression vectors leads to the surface expression of HLA-DR1, because the allelic polymorphism of HLA-DR is known to be conferred by the β chain while the α chain is monomorphic (65). Flow cytometric analysis was next performed to verify the proper surface expression of HLA-DR1 on transiently transfected 293T cells. As depicted in Fig. 1, HLA-DR1 could not be detected on 293T cells transfected only with pHXB-Luc (Fig. 1A), while the great majority of 293T cells (95% of positive cells) cotransfected with pHXB-Luc, pRSV.5 *neo* DRα, and pRSV.3 DR1β expressed high levels of surface HLA-DR1 (mean fluorescence value, 32.3) (Fig. 1B). Staining of intracellular viral p24 antigen was also performed on the same transfected population of 293T cells. Comparable high percentages of p24-positive transfected cells were seen following both transfection solely with pHXB-Luc (89% of positive cells) (Fig. 1C) and cotransfection with pHXB-Luc, pRSV.5

TABLE 1. Detection with immunomagnetic beads of host-derived HLA-DR1 glycoproteins acquired by HXB-Luc particles

Virus preparation	Capture of HIV-1[a] with magnetic beads coated with:	
	Anti-CD3 (OKT3)	Anti-HLA-DR (2.06)
HXB-Luc HLA-DR1/NEG	17.3 ± 0.8	36.8 ± 3.8
HXB-Luc HLA-DR1/POS	20.3 ± 0.8	165.8 ± 15.6

[a] Results shown are the means ± standard deviations of triplicate samples. Values are expressed in picograms of p24.

neo DRα, and pRSV.3 DR1β (88% of positive cells) (Fig. 1D). Data from these studies suggest that the likelihood that an HLA-DR1-expressing 293T cell also actively produces HXB-Luc particles is high considering that the percentage of cells expressing surface HLA-DR (95%) is greater than the percentage of virus-expressing cells (88%). Therefore, a high percentage of HXB-Luc particles will be shed from HLA-DR1-positive 293T cells.

Detection of physically associated host HLA-DR1 on HXB-Luc particles. To ensure that virions harvested from HLA-DR1-expressing 293T cells had properly acquired this specific host cell surface component, virus capture studies were conducted with magnetic beads. This technique relies on the use of magnetic beads coated with an antibody specific for the molecule of interest. After several washes of the magnetic beads, captured viruses are detected by monitoring the p24 content with an enzymatic assay. In our test, magnetic beads were coated with the monoclonal antibody 2.06, which is directed at a monomorphic epitope on HLA-DR. In parallel, as a negative control, we used magnetic beads coupled to OKT3, an antibody specific for a molecule not found on the surface of 293T cells (data not shown).

As depicted in Table 1, background levels of captured viruses were obtained when we used OKT3-coupled magnetic beads (17.3 pg of p24 when HXB-Luc HLA-DR1/NEG was used and 20.3 pg of p24 when HXB-Luc HLA-DR1/POS was used). Magnetic beads coated with 2.06 inefficiently captured progeny virus harvested from 293T cells transfected only with pHXB-Luc (36.8 pg of p24 with HXB-Luc HLA-DR1/NEG). However, 2.06-coupled magnetic beads efficiently captured viruses produced by HLA-DR1-positive HIV-1-producing 293T cells that had been cotransfected with pHXB-Luc, pRSV.5 *neo* DRα, and pRSV.3 DR1β (165.8 pg of p24 when HXB-Luc HLA-DR1/POS was used). Altogether, results from this set of experiments indicate that host-derived HLA-DR1 glycoproteins are physically present on the surface of HXB-Luc particles produced by HLA-DR1-positive, transfected 293T cells. Therefore, this transient-expression system is appropriate for the production of HIV-1 particles bearing or not bearing virally embedded host cell membrane HLA-DR glycoproteins.

Infection of susceptible cells with HXB-Luc leads to a single-round infection event. To accomplish transcription of the *luciferase* reporter gene encoded by HXB-Luc, not only does the virus have to enter the target cell, but the virus genomic material has to integrate itself within the host chromosome. Therefore, the detection of a luciferase signal is indicative of an almost complete HIV-1 replicative cycle. We initially tested recombinant luciferase-encoding HIV-1 particles on Jurkat-*tat*, a Jurkat E6-1 derivative that stably expresses the viral transactivator protein Tat (61). This cell line was used because the presence of the Tat protein in the target cell strongly upregulates the transcription of the virus-encoded *luciferase* gene and, as a result, leads to higher levels of luciferase activity. Moreover, Jurkat-*tat* cells are negative for cell surface expression of HLA-DR (data not shown), implying that progeny virus budding out from these cells will not incorporate such host-derived components. This property is of prime importance, as virus particles produced after the infection of Jurkat-*tat* cells with HXB-Luc will not acquire HLA-DR glycoproteins. The absence of cellular HLA-DR on the surface of HIV-1 is crucial, because there is a possibility that virally incorporated host-derived HLA-DR participates in the initial events of the viral replicative cycle.

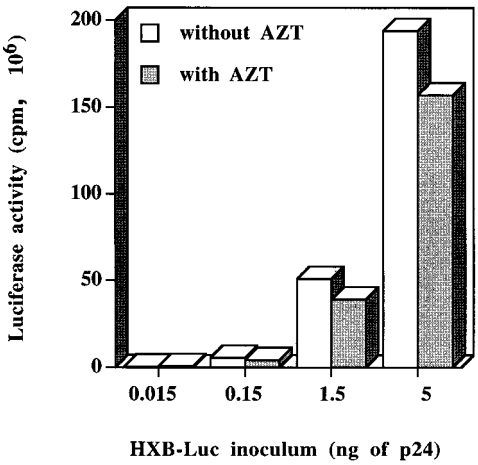

FIG. 2. HXB-Luc-mediated luciferase activity following infection of Jurkat-*tat* cells in the absence or presence of AZT. Jurkat-*tat* cells were infected with increasing amounts of HXB-Luc (0.015, 0.15, 1.5, and 5 ng of p24). Reinfection events were blocked by adding to the culture medium 1 μM AZT at 24 h after virus infection. Cells were lysed at 72 h postinfection, and luciferase activity was monitored. Data shown represent the means of triplicate samples. All standard deviations were within 10% of the means.

To determine if inoculation of susceptible cells with HXB-Luc leads to a single-round infection event, virus infection was performed in the presence or absence of 1 μM AZT, which was added 24 h postinfection. The use of this antiviral drug was aimed at abrogating reinfection phenomena. Determination of luciferase activity following lysis of cells infected with increasing concentrations of virions revealed that levels of luciferase activity were dependent on the amount of HXB-Luc used for infection (0.015, 0.15, 1.5, and 5 ng of p24), therefore suggesting that the measured signal is indeed the result of virus infection (Fig. 2). This fact is further reinforced by the observation that the process of HXB-Luc infection was almost completely abolished following preincubation with 200 μg of pooled purified IgG from HIV-1-seropositive patients/ml (data not shown). The frequency of reinfection events was found to be minimal, since the inclusion of AZT at 24 h following the infection of Jurkat-*tat* cells with HXB-Luc resulted only in a 19 to 23% decrease of luciferase activity, depending on the initial virus inoculum. Therefore, we considered the reinfection phenomena to have no significant impact on our subsequent studies.

HIV-1 infectivity is enhanced by the presence of virally incorporated host cell membrane HLA-DR1. Since the only difference between the two HXB-Luc preparations was the incorporation or lack of incorporation of host-derived HLA-DR1 (HXB-Luc HLA-DR1/POS and HXB-Luc HLA-DR1/NEG), any changes in the biological properties of such virions would be directly or indirectly due to the presence of these virally embedded host-derived glycoproteins. We then com-

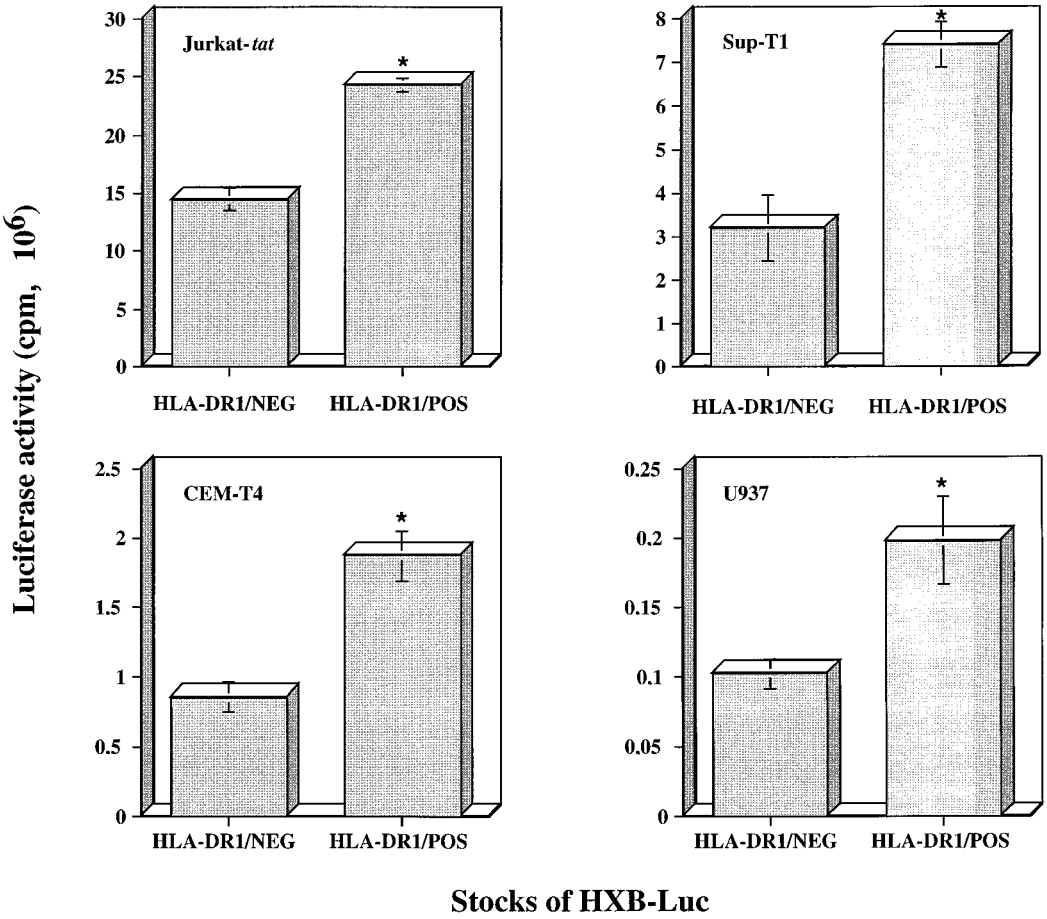

FIG. 3. Infectivities of HLA-DR1-negative and -bearing HXB-Luc particles with, as targets, several lymphoid cell lines. Jurkat-*tat*, Sup-T1, CEM-T4, and U937 cells were infected with either HXB-Luc HLA-DR1/NEG or HXB-Luc HLA-DR1/POS virus (1.5 ng of p24) and were incubated for 72 h at 37°C. Luciferase activities were next monitored in lysed cells. Results shown are the means ± standard deviations of triplicate samples. Asterisks indicate significant differences from values for infection with HLA-DR1/NEG particles ($P < 0.01$).

pared, in different HIV-1-susceptible CD4-positive cell lines, the infectiousness of HXB-Luc particles with or without cellular HLA-DR1 glycoproteins. Infection experiments were performed with equal amounts of each virus stock normalized according to its p24 content, and luciferase activity in cell lysate was assessed after a 72-h incubation at 37°C. A 1.7- to 2.3-fold increase in virus infectivity, as determined by monitoring luciferase activity, was seen when Jurkat-*tat*, Sup-T1, and CEM-T4 cells were infected with HLA-DR1-bearing viruses compared to infectivity with HXB-Luc devoid of host-derived HLA-DR1 glycoproteins (Fig. 3). A similar enhancement in the process of virus infection was noticed when the promonocytoid cell line U937 was used as a target (1.9-fold increase). It should be noted that increases in virus infectivity were all statistically significant ($P < 0.01$). These results indicate that the potentiating effect on the process of virus infection, which is mediated by virally incorporated cellular HLA-DR1 glycoproteins, is not cell type specific.

To ascertain that the enhancement of HIV-1 infectivity conferred by HIV-1-associated host-derived HLA-DR1 glycoproteins is not an isolated epiphenomenon that might be related to virus stocks used for these particular studies, further experiments were performed with virus preparations originating from three independent transfections. Each cell-free virus stock was collected from transfection experiments carried out on different days, and infectivity was assessed with Jurkat-*tat* cells by following the procedure described above. Infectivity of HLA-DR1-bearing virions was still superior to that demonstrated by HXB-Luc particles free of host HLA-DR1 glycoproteins (1.6- to 2.3-fold increase) (Fig. 4). These differences at the level of HIV-1 infectivity were statistically significant, with a P value of less than 0.01.

Kinetics of HIV-1 infection is modulated by the acquisition of host-derived HLA-DR1 glycoproteins. Next, we wondered if the presence of host cell membrane HLA-DR1 on HIV-1 could modify the rapidity with which virus infection proceeds. A diminution in the overall period of time necessary to achieve virus binding and entry would enable the virus to infect host cells more rapidly and would be very advantageous for circulating virions present in bodily fluids. The kinetics of virus infection was monitored with Jurkat-*tat* cells infected with HXB-Luc stocks, and infection was terminated with the use of pooled purified human IgG from HIV-1-seropositive patients. At all studied time periods, HLA-DR1-bearing particles showed kinetics of infection more rapid than that of HXB-Luc devoid of host-derived HLA-DR1 molecules (Fig. 5A). The kinetics of HIV-1 infection was also more rapid at earlier time points following virus inoculation (Fig. 5B). Indeed, a 1.6- to 2.0-fold increase in HIV-1 infectivity was already seen between 5 and 15 min postinfection, and this potentiating effect, which

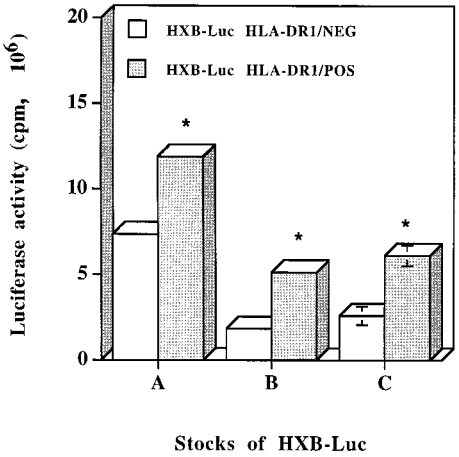

FIG. 4. Infectivities of HXB-Luc particles bearing or not bearing host-derived HLA-DR1 produced by three independent transfections. Jurkat-*tat* cells were infected with either HXB-Luc HLA-DR1/NEG or HXB-Luc HLA-DR1/POS virus (1.5 ng of p24) originating from independent transfections and were incubated for 72 h at 37°C. Infected cells were lysed, and luciferase activities were assessed. The data presented are the means of triplicate samples. Error bars represent sample standard deviations. Asterisks indicate significant differences from values for infection with HLA-DR1/NEG particles ($P < 0.01$).

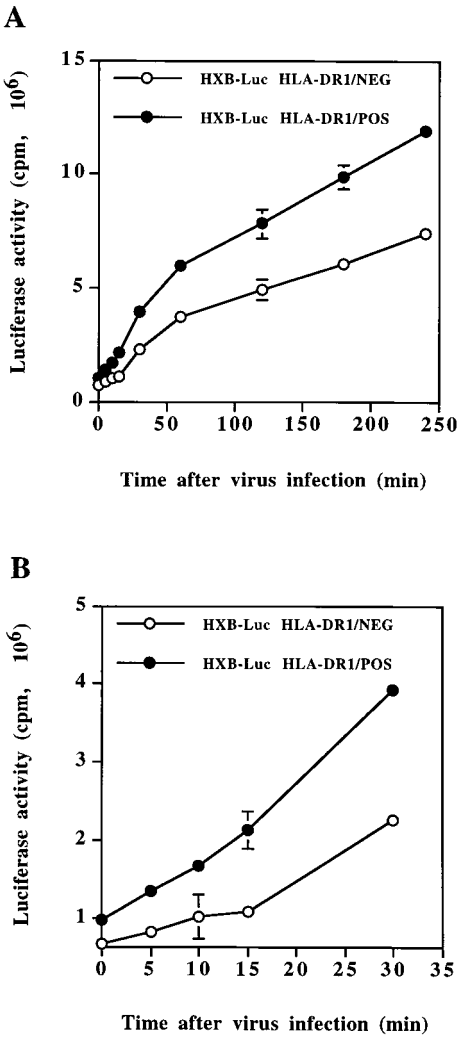

FIG. 5. Kinetics of virus infection when virions bearing or not bearing host-derived HLA-DR1 were used. Jurkat-*tat* cells were infected with either HXB-Luc HLA-DR1/NEG or HXB-Luc HLA-DR1/POS virions (1.5 ng of p24) and, at specific time points, infection was terminated by the addition of human anti-HIV-1 neutralizing antibodies. Cells were left for 72 h at 37°C and were next lysed before determination of luciferase activity. The values plotted represent the averages of triplicate samples ± the standard deviations.

is mediated by the incorporation of host-derived HLA-DR1, was similar to the one detected at a later time point (4 h) after the initial virus inoculation (1.6-fold increase). These data suggest that virally embedded host cell HLA-DR1 positively affects early events in the viral life cycle.

Enhancement of HIV-1 infectivity is still observed in primary target cells. To more closely parallel physiological conditions, similar infection studies were performed with primary PBMCs isolated from a healthy, seronegative donor. A 1.8- to 2.1-fold increase in HIV-1 infectivity was seen in PBMCs infected with two different inocula of HXB-Luc particles carrying on their surfaces host HLA-DR1 compared to infection with HLA-DR1-free virions (Fig. 6A and B).

DISCUSSION

The main objective of this study was to evaluate the putative role played by virally acquired host cell membrane HLA-DR in the life cycle of HIV-1. Keeping in mind that the major cellular receptor for HIV-1, the CD4 molecule, is also the natural ligand of HLA-DR, we postulated that the interaction between virally embedded host HLA-DR and surface CD4 could take place during the infection process. The observation that high levels of host cellular HLA-DR molecules are found embedded within HIV-1 (1, 7, 51) represents another factor that prompted us to initiate these investigations.

For our studies, we used the recombinant luciferase-encoding infectious molecular clone pHXB-Luc, which carries the *Photinus pyralis* reporter *luciferase* gene in place of the viral *nef* gene. Data from our studies indicated that HXB-Luc particles, which lead to the expression of firefly luciferase upon infection of susceptible cells, can be used as a highly sensitive system to measure single-round infection events. This property enabled us to precisely quantify the extents of HIV-1 infection in several cell lines and, more importantly, to assess whether the acquisition of host-derived HLA-DR glycoproteins by HIV-1 influences virus infectivity. Generation of HIV-1 particles harboring cellular HLA-DR1 on their surfaces was achieved by cotransfecting 293T cells with pHXB-Luc and eukaryotic expression vectors encoding the α and β chains of HLA-DR1 heterodimeric glycoprotein. Progeny viruses devoid of such host-derived components were produced following transfection with pHXB-Luc exclusively. As a result, we had in our hands virus preparations of the same strain of HIV-1 that differed only in the presence or absence of virally embedded cellular HLA-DR1.

With these tools, we were able to demonstrate that the presence of host-derived HLA-DR-1 on HIV-1 leads to a 1.6- to 2.3-fold increase in HIV-1 infectivity, depending on the cell line tested. This phenomenon was not cell type specific, as a similar enhancement of HIV-1 infectivity, mediated by the presence of host-derived HLA-DR1 on HIV-1 particles, was seen in three different T-lymphoid cell lines (Jurkat-*tat*, SupT1, and CEM-T4) and in premonocytoid U937 cells. We demonstrated that the acquisition of host-derived HLA-DR1 also increases HIV-1 infectivity in mitogen-stimulated PBMCs. Studies aimed at evaluating kinetics of virus infection revealed that the influence of host-derived HLA-DR1 on HIV-1 infec-

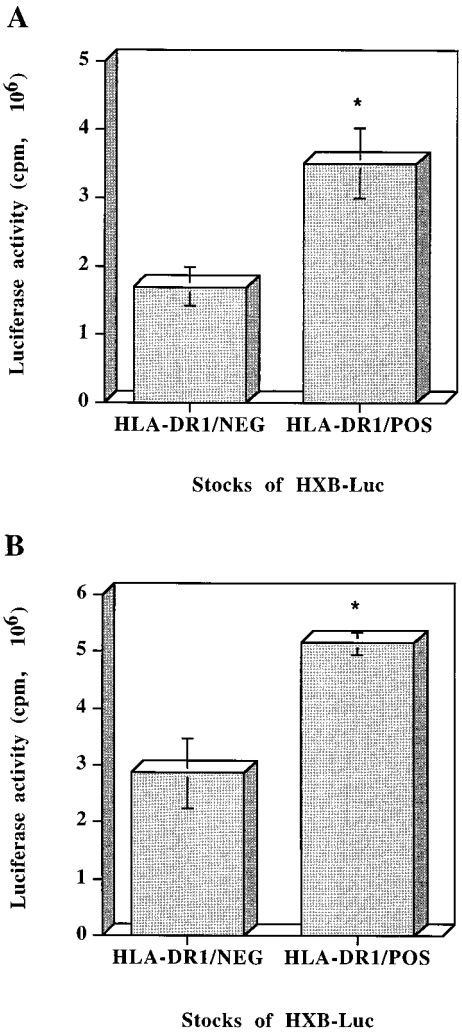

FIG. 6. Infectivities of HXB-Luc particles bearing or not bearing host-derived HLA-DR1 with, as targets, primary PBMCs. An HXB-Luc HLA-DR1/NEG or HXB-Luc HLA-DR1/POS stock was used to infect PHA-stimulated PBMCs (1.5 ng of p24 [A]; 3 ng of p24 [B]). Cells were lysed after 72 h of infection, and luciferase activities were evaluated. Average data ± standard deviations from triplicate samples are shown. Asterisks indicate significant differences from values for infection with HLA-DR1/NEG particles ($P < 0.01$).

tivity is most likely due to an enhancement of the overall virus-binding avidity, resulting in positive effects on earlier events in the virus replicative cycle, such as adsorption and penetration steps. Altogether, these data suggest that virally incorporated host-derived HLA-DR1 glycoproteins are biologically active since they enhance the infectivity of HIV-1 particles.

We believe that the observed upregulation of HIV-1 infectivity is most likely mediated by associations between virally embedded cellular HLA-DR and surface CD4 on the target cell. Although the force of interaction between these two molecules is relatively low ($K_d = 3.2 \times 10^{-6}$ M for HLA-DR4-CD4) (6) when compared, for example, to the affinity constant for the gp120-CD4 interaction ($K_d = 4 \times 10^{-9}$ M) (44), the previously reported large amount of HIV-1-embedded host HLA-DR (1, 7, 51) may compensate for this weaker dissociation constant. Thus, the moderate, but constant, increase in HIV-1 infectivity that we detected in this work (1.6- to 2.3-fold) is possibly related to the weak affinity of HLA-DR for binding to its CD4 counterreceptor. Nonetheless, we feel that these additional virus-cell interactions, although weaker than that of gp120 and CD4, may result in a kind of "velcro effect" where the sum of virion-bound host-derived HLA-DR and surface CD4 interactions result in a stronger attachment of the virus to its target. This effect may provide an advantage to the virus, enabling it to anchor more firmly the target cell. The virus may then stick to the target cell and roll on the surface of the cytoplasmic membrane until the appropriate conformation is attained, thereby allowing the viral gp41 to initiate and mediate the fusion process. It is already known that HIV-1 can remain on the surface of susceptible cells for a long period of time in vitro (64). Furthermore, virally acquired host HLA-DR and surface CD4 interactions would help the viral entity to overcome the negative electrostatic repulsion force between cell and virus membranes (21).

In this work, we have not tried to compare the infectivities of virions bearing or not bearing HLA-DR1 on primary monocyte-derived macrophages. This is because the molecular clone pHXB-Luc encodes the external envelope protein gp120 of IIIB, a strain of HIV-1 that does not replicate well in monocyte-derived macrophages (31). We are presently constructing a recombinant luciferase-encoding macrophage-tropic molecular clone of HIV-1 that will permit us to evaluate whether the presence of host-derived HLA-DR on progeny virus can affect virus infectivity in monocyte-derived macrophages. These studies are needed, since monocytes/macrophages are also infected in vivo with HIV and are thought to play a key role in the pathogenesis of the disease (4, 15, 56). Moreover, virally infected monocytes/macrophages are potent producers of HLA-DR-bearing virions because they are known to express on their surfaces all MHC-II isotypes (DR, DP, and DQ). It should be noted that the use of immunomagnetic beads has revealed that host-derived HLA-DR glycoproteins are found embedded within HIV-1_{Ada-M}, HIV-1_{JR-CSF}, and HIV-1_{JR-FL} grown on primary monocyte-derived macrophages (unpublished observations).

Recently, we reported that the relative amounts of host-derived HLA-DR acquired by laboratory isolates of HIV-1 grown on lymphoid cell lines and by HIV-1 clinical strains produced by primary PBMCs are quantitatively influenced by the virus strain and producer cell line (7). We believe that these properties may influence both viral transmission and the outcome of the disease. First, a viral strain from a donor that carries high levels of host-derived HLA-DR may result in increased infectivity. For example, a more efficient infection of macrophages located in the mucosa will result in a more important dissemination rate of the virus, as these cells are considered to be the first cell type to be infected at the time of transmission (36, 48, 59). A high density of cell-derived HLA-DR glycoproteins on the surfaces of progeny virions could then positively affect spreading of HIV-1 between infected and uninfected subjects. Second, many HIV-1 variants called quasispecies arise during the course of the disease due to the high mutation rate of the virus (14). It is possible that, among quasispecies, variants of HIV-1 that possess the capacity to acquire elevated levels of host-derived HLA-DR are selected. On the basis of our findings, the production of virions bearing a high number of cellular HLA-DR on their surfaces will affect the virus' biological cycle and may influence the pathogenesis of the disease.

We previously postulated that the rate of HLA-DR incorporation within budding HIV-1 particles may depend upon the HLA-DR allele (7). Therefore, the genetic status of the infected individual may affect the level of virally embedded cellular HLA-DR. This work focused on a single HLA-DR allele,

namely HLA-DR1. The facts that the HLA-DR locus is highly polymorphic and that the affinity between HLA-DR and CD4 may vary according to the HLA-DR allele (22) suggest that some specific virally incorporated HLA-DR alleles may lead to a greater increase in HIV-1 infectivity. We are in the process of evaluating such a possibility by generating HIV-1 particles bearing on their surfaces different HLA-DR alleles.

As suggested before, the incorporation of host-derived HLA-DR by HIV-1 may contribute to disease progression if we suppose that the acquisition of HLA-DR alleles with high affinities for CD4 is associated with a concomitant increase in HIV-1 infectivity. Epidemiological studies seem to support this assumption, because some reports have provided evidence for an association between MHC alleles or haplotypes and disease progression (35, 66). More precisely, the haplotype A1-B8-DR3 and the alleles HLA-DR2 and HLA-DR5 have been associated with an accelerated progression to AIDS (19, 33, 34, 40). Another example is the relation between the HLA-DR1 allele and Kaposi's sarcoma or the correlation between HLA-DR3 and the apparition of opportunistic infections (39). The rapid decline of CD4-expressing T cells in seropositive hemophiliacs was shown to be linked with certain HLA phenotypes, such as HLA-A9, -DR1, and -DR3 (52).

In summary, we have demonstrated that the presence of host HLA-DR1 within HIV-1 can enhance its infectivity toward susceptible cells. This observation is highly relevant for in vivo situations where the presence of HLA-DR-bearing virions may affect the efficiency of transmission and the development of AIDS. Host-derived proteins acquired by HIV-1 during the budding process should thus be considered key elements in viral pathogenicity. Further studies are needed to assess whether the biology of HIV-1 may be modulated by the HLA-DR polymorphism.

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R.C. and J.-F.F. contributed equally to this work.

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