The Amount of Host HLA-DR Proteins Acquired by HIV-1 Is Virus Strain- and Cell Type-Specific

RÉJEAN CANTIN, 1 JEAN-FRANC¸OIS FORTIN, 1 and MICHEL TREMBLAY 2

Centre de Recherche en Infectiologie, Centre Hospitalier de l’Université Laval, and Département de Microbiologie, Faculte´ de Me´decine, Universite´ Laval, Ste-Foy, Quebec, Canada G1V 4G2

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We semiquantitatively monitored the incorporation of host membrane proteins on different strains of human immunodeficiency virus type 1 (HIV-1) grown in several human CD4+ lymphoid cell lines and in primary mitogen-stimulated peripheral blood mononuclear cells. The relative amounts of virally acquired cell proteins were estimated by the ability of HIV-1 to be captured by magnetic beads coated with monoclonal antibodies. Here we report that, among host surface proteins studied, HLA-DR molecules were the most abundant virion-bound host molecules. We have also found that, in contrast to previous studies, HLA-DP and -DQ isotypes were also present on virus progeny. More importantly, we determined that the relative levels of virally acquired host HLA-DR proteins, as estimated by capture with immunomagnetic beads, greatly differed depending on the virus strain and the producer cell. These observations extend beyond already published results and suggest that the process of incorporation of cellular molecules on newly released virus particles is a phenomenon that relies on both the virus strain and producer cell line. These in vitro observations are of prime importance considering that virus-acquired host molecules have been recently shown to affect the biology of HIV.

INTRODUCTION

Infection of susceptible cells with the human immunodeficiency virus (HIV) occurs via interaction between the external viral envelope glycoprotein gp120 and the CD4 molecule displayed on the host cell surface (Dalgleish et al., 1984; Klatzmann et al., 1984). In addition to this virally encoded surface protein, whose function has been extensively studied, other molecules have been demonstrated to be present on the virus surface. The incorporation of cellular constituents on virus particles has been reported to occur in retroviruses as they bud from infected cells (Azocar and Essex, 1979; Bubbers and Lilly, 1977; de The` et al., 1964; Gelderblom et al., 1987a; Lando et al., 1983; Lee et al., 1982). These host constituents, depending on their nature, may directly or indirectly participate in early events in the virus replicative cycle. It has been shown that acquisition of cellular proteins by budding viruses is a selective process. In fact, specific cellular proteins were detected on virions such as MHC-I molecules on murine leukemia virus (Bubbers and Lilly, 1977), leukocyte alloantigens on feline leukemia virus (Lee et al., 1982), and receptors for interleukin-2 on human T-lymphotropic virus type-I (Lando et al., 1983). It has been postulated that the selective incorporation of cellular antigens within retroviruses envelope may affect the host range and influence the course of the disease following infection (Lando et al., 1983; Lee et al., 1982).

Similar studies were extended to HIV and it was shown that this retrovirus could also incorporate several cell membrane proteins including HLA-DR, β2-microglobulin, ICAM-1, LFA-1, CD43, CD44, CD63, and the transferrin receptor (CD71) (Arthur et al., 1992; Benkirane et al., 1994; Capobianchi et al., 1994; Castilletti et al., 1995; Fais et al., 1995; Gelderblom et al., 1987a; Henderson et al., 1987; Hoxie et al., 1987; Meerloo et al., 1992, 1993; Orentas and Hildreth, 1993; Schols et al., 1992). For example, Arthur et al. (1992) have reported that the β2-microglobulin and the α and β chains of MHC-II (DR-isotype) were physically associated with the surface of HIV-1, HIV-2, and SIV. An interesting observation of some of these studies was that HLA-DR was the only isotype of major histocompatibility complex class-II molecules (MHC-II) found embedded within HIV-1 particles, as HLA-DP and HLA-DQ were excluded from the progeny virus (Arthur et al., 1992; Henderson et al., 1987; Hoxie et al., 1987).

Recent studies have provided evidence suggesting that virion-embedded host proteins can affect the biology of the virus (Gomez and Hildreth, 1995; Lee Guo and Hildreth, 1995; Saiffudin et al., 1995). The amount of virally acquired cellular molecules could therefore have an impact on the virus activity. In order to supplement our knowledge with regard to the incorporation of cellular constituents on budding HIV-1 particles, we analyzed different strains of HIV-1 harvested from various CD4+ leukemic cell lines and from primary PHA-stimulated mono-

1 Contributed equally to this work.

2 To whom correspondence and reprint requests should be addressed. Fax: (418) 654-2715. E-mail: tremblam@vm1.ulaval.ca.
nuclear cells. The relative levels of host membrane proteins incorporated by progeny virus were investigated by monitoring the ability of HIV-1 to be captured by immunomagnetic beads. This technique allowed us to determine the proportion of viruses that expresses on their surface the threshold number of host molecules required to achieve an efficient immunomagnetic isolation. In this study, we show that incorporation of cell-derived HLA-DR proteins on HIV-1 is a phenomenon that is quantitatively modulated by the virus and cell type. This report is the first to demonstrate that the process of acquisition of host proteins by HIV is influenced by both the virus and the producer cell.

MATERIALS AND METHODS

Cells

Cells were maintained in complete culture medium made of RPMI-1640 supplemented with 10% fetal calf serum, glutamine (2 mM), penicillin (100 U/ml), and streptomycin (100 mg/ml). RAJI cells, obtained from the American Type Culture Collection (Rockville, MD), have been previously shown to express very high levels of cell surface HLA-DR, -DP, and -DQ proteins (Accolla, 1983). RAJI cells were rendered susceptible to HIV-1 infection by transfecting them with the human CD4 cell surface glycoprotein using an amphotropic retrovirus as described previously (Tremblay et al., 1994). In brief, CD4 was introduced into RAJI cells by infection with an amphotropic retrovirus vector that contained the human CD4 cDNA driven by the cytomegalovirus enhancer/promoter region and the neo gene driven by the simian virus 40 promoter. Stable transfectants of RAJI-CD4 cells were cultured in complete culture medium supplemented with 1 mg/ml of the selective agent G418 (GIBCO - BRL, Gaithersburg, MD). The lymphoblastoid CD4+ T-cell lines H9, HUT 78, and Molt 4 clone 8 were provided by the AIDS Research and Reference Reagent Program, Division of AIDS, NIAID, NIH (Rockville, MD).

Antibodies

Anti-CD4 (anti-Leu-3A, clone S53) was purchased from Becton–Dickinson Immunocytometry (San Jose, CA). Anti-CD3 (clone OKT3) (Van Wauwe et al., 1980), anti-MHC-I (clone W6/32) (Brodsky and Parham, 1982), and anti-HLA-DR (clone 2.06) (Charron and McDevitt, 1979) monoclonal antibodies were obtained from the American Type Culture Collection (Rockville, MD). Anti-HLA-DP (clone B7.21) (Watson et al., 1983) and anti-HLA-DQ (clone BT3.4) (Accolla et al., 1982) were kindly provided by Dr R.-P. Sékaly (Institut de Recherches Cliniques de Montréal, Montréal, Que., Canada). Anti-HLA-DR, -DP, and -DQ antibodies are directed against monomorphic regions of each molecule. Anti-CD54 (clone RR1/1.1.1) was generously supplied by Dr R. Rothlein (Boehringer Ingelheim Pharmaceuticals, Inc., Ridgefield, CO). Antibodies were isolated from hybridoma culture supernatants and purified by recombinant protein A/G-Sepharose affinity chromatography.

Production of virus particles

Virus stocks were prepared from acutely infected cells. In brief, human lymphoid cell lines (RAJI-CD4, H9, HUT 78, and Molt 4 clone 8) were infected with various strains of HIV-1 (LAI, IIIRF, and NL4-3) at a multiplicity of infection of 0.01 (infectious particle/target cell). At the maximal virus production and before extensive cytopathic effects were seen, cells were centrifuged at 300 g for 5 min and the virus-containing supernatant was clarified at 2000 g for 30 min and was filtered through a 0.45-μm cellulose acetate membrane to remove cellular debris. Thereafter, the virus-containing supernatant was stored at −80° in aliquots. HIV-1_LAI was initially harvested from latently infected ACH-2 cells following treatment with TNF-α. The IIIRF strain of HIV-1 was kindly provided by Dr. R. C. Gallo, as cell-free supernatant from infected H9 cells, through the AIDS Research and Reference Reagent Program. Stock of the NL4-3 strain of HIV-1 was prepared by transfecting plasmid DNA into Sup-T1, a CD4+ T-cell line highly susceptible and permissive to HIV-1 replication (unpublished observations). Transfection of pNL4-3 was achieved by electroporation according to a standard protocol (Cann et al., 1988). The pNL4-3 molecular clone of HIV-1 was provided by the AIDS Research and Reference Reagent Program. Clinical isolates HIV-1_334, HIV-1_336, and HIV-1_438 have been described previously (Tremblay et al., 1990) and were produced on phytohemagglutinin-stimulated peripheral blood cells isolated from the same healthy donor. The percentage of pelleted virus-associated p24 was evaluated by subjecting each virus stock to ultracentrifugation (Heraeus type HTA 13.8 rotor; 12,000 rpm; 90 min at 4°C).

Detection of cellular molecules incorporated on HIV-1 by immunomagnetic beads

Different mouse monoclonal antibodies (OKT3, OKT4, RR1/1.1.1, W6/32, 2.06, B7.21, and BT3.4) were bounded to BioMag anti-mouse IgG particles (Fc specific; PerSeptive Diagnostics, Inc., Cambridge, MA). Briefly, magnetic particles were washed four times using a magnetic separation unit with binding buffer made of phosphate-buffered saline (PBS), pH 7.2, supplemented with 0.1% bovine serum albumin and 0.02% thimerosal. Thereafter, washed magnetic particles (2.5 × 10⁶) were incubated overnight at 4°C using gentle mixing with 40 μg of each antibody. Antibody-coated magnetic particles were washed four times with binding medium and were stored at 4°C until used. Similar amounts of each virus stock, standardized by their p24 content (2500 pg), were incubated with antibody-coated magnetic particles (12.5 ×
10^6) for 60 min at 4°C under gentle mixing. Complexes consisting of immunomagnetic particles and viruses were washed four times with binding medium and were resuspended in 100 μl of binding medium. Samples were immediately tested for their p24 content by a commercial enzymatic assay.

The reliability of the present immunomagnetic capture of HIV-1 for quantitative measurements of virion-bound host proteins was determined by incubating a fixed amount of HIV-1 (2500 pg of p24, strain HIV-1^NL4-3 grown in RAJI-CD4 cells) with increasing concentrations of anti-HLA-DR-coated magnetic beads (1.25, 2.5, 6.25, 12.5, 18.75, and 25 × 10^6). The amount of viruses captured was found to be directly proportional to the amount of antibody-coated beads reaching a plateau when using 12.5 × 10^6 antibody-coated magnetic particles. For example, results from one representative experiment demonstrate that 100, 170, 540, 1110, 1100, and 1075 pg of p24 were captured with the use of 1.25, 2.5, 6.25, 12.5, 18.75, and 25 × 10^6 anti-HLA-DR-coated magnetic beads, respectively. Similar standardization experiments were carried out for each monoclonal antibody used in our study. Furthermore, results obtained with magnetic beads coated with OKT3 were used as negative control values because studied cell lines were all negative for cell surface CD3 expression (data not shown). Values with anti-CD3-coated beads were consistently <10 pg of p24 and have been subtracted from the values shown for other antibodies. Capture of HIV-1^NL4-3 (2500 pg of p24) harvested from RAJI-CD4 cells with anti-HLA-DR-coated magnetic beads gave intraassay and interassay variations of 8 and 9%, respectively, suggesting that this assay is reproducible (data not shown). Preliminary experiments indicated that, in our hands, capture of HIV-1 particles with immunomagnetic beads gave both lower background levels of p24 and better reproducibility than the previously described virus capture assay, which relies on the use of ELISA plates coated with specific antibodies (Orentas and Hildreth, 1993).

**p24 enzymatic assays**

Virus stocks were quantitated by measuring the amount of the major viral core p24 protein with the use of a commercial enzymatic test (Organon Teknika, Durham, NC).

**Flow cytometry analysis**

Levels of surface molecules were detected by indirect immunofluorescence using a cytofluorimeter. Briefly, 1 × 10^5 cells were first incubated with an experimentally determined saturating concentration of an appropriate murine monoclonal antibody for 30 min on ice. Samples were washed twice in PBS and incubated for 30 min on ice with a R-PE-conjugated goat anti-mouse IgG. After two washes with PBS, samples were fixed with 1% (w/vol) of paraformaldehyde and analyzed by a cytofluorimeter. Controls consisted of commercial isotype-matched murine monoclonal antibodies (Sigma, St. Louis, MO).

**Statistical analysis**

Statistically significant differences between groups were performed with the analysis of variance (ANOVA) module of SAS software (version 6.07, SAS Institute, Cary, NC) using the Duncan’s multiple range test. P values õ 0.05 were considered statistically significant (P values are given in the figure legends). All data are presented as mean ± SEM.

**RESULTS**

Immunomagnetic recovery of viruses harvested from acutely infected lymphoid cells

Magnetic beads coated with specific monoclonal antibodies were used to detect cell membrane proteins associated with HIV-1 particles budding from acutely infected cells. First, we have used viruses harvested from a lymphoblastoid B-cell line that is highly susceptible to HIV-1 infection. RAJI-CD4 cells were inoculated with HIV-1^NL4-3 and cell-free supernatant was harvested before virus-mediated cytopathic effects were seen. It is of interest to note that extensive cytopathic effects are induced in RAJI-CD4 cells by the majority of laboratory strains of HIV-1, including NL4-3, IIIRF, and LAI (data not shown).

Results from Table 1 indicate that progeny virus was captured efficiently by magnetic beads coated with monoclonal antibodies specific for several cell surface molecules. Comparable recovery rate of virus particles was achieved with the use of magnetic beads coated with antibodies against the adhesion molecule ICAM-1 (CD54), MHC-I, and two determinants of MHC-II (HLA-DR and HLA-DQ). Magnetic beads coated with a monoclonal antibody directed against a monomorphic epitope of the HLA-DR determinant of MHC-II (clone 2.06) were the most efficient to capture viruses harvested from RAJI-CD4 cells.

**TABLE 1**

<table>
<thead>
<tr>
<th>Antibody (clone)</th>
<th>Core protein p24</th>
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<tbody>
<tr>
<td>CD3 (OKT3)</td>
<td>5</td>
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<tr>
<td>CD4 (OKT4)</td>
<td>5</td>
</tr>
<tr>
<td>CD54 (RR1/1.1.1)</td>
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</tr>
<tr>
<td>MHC-I (W6/32)</td>
<td>36</td>
</tr>
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<td>MHC-II/HLA-DR (2.06)</td>
<td>1220</td>
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<tr>
<td>MHC-II/HLA-DP (BT.21)</td>
<td>64</td>
</tr>
<tr>
<td>MHC-II/HLA-DQ (BT3.4)</td>
<td>64</td>
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* Results are the mean of three determinations. All standard deviations were within 10% of the mean. Values shown are expressed in picograms of p24.
CD4 cells. Virus particles recovered with immunomagnetic particles were shown to be infectious when cultured on Sup-T1 cells and the extent of virus yield correlated with the amount of captured virus (data not shown).

A background level of virus recovery was seen with a monoclonal antibody specific for CD4, indicating that this cell surface molecule is not acquired by budding virus progeny. The failure to recover HIV-1 particles with this antibody was not associated with changes in its binding affinity since all cell lines tested in our studies (RAJI-CD4, HUT 78, H9, and Molt 4 clone 8) could be efficiently captured with magnetic beads coated with the same anti-CD4 antibody (data not shown). Furthermore, magnetic beads coated with anti-CD54, -MHC-I, -HLA-DR, -DP, and -DQ antibodies demonstrated also the same capacity to immunoselect studied cell lines expressing the appropriate epitope, suggesting that immunomagnetic beads used in our assay share a similar ability to capture cells and, most likely, to bind to virus particles carrying the antibody-specific host protein.

The level of host HLA-DR acquired by HIV-1 is virus strain- and cell type-specific

Following the demonstration of the reliability and reproducibility of virus capture by immunomagnetic beads, comparative analysis was next carried out to semiquantitatively monitor the relative levels of viruses expressing on their surface the threshold number of virally embedded cellular proteins needed to be captured by immunomagnetic beads. Three different laboratory isolates of HIV-1 were harvested from acutely infected RAJI-CD4 cells and immunomagnetic separation of such virus particles was achieved by incubating together magnetic beads coated with specific monoclonal antibodies with similar amounts of each virus stock standardized by their p24 content. Magnetic beads coated with antibodies specific for ICAM-1, MHC-I, HLA-DR, and HLA-DQ proteins captured HIV-1LaA, HIV-1B, and HIV-1NL43 with an almost comparable efficiency (Fig. 1). However, the amount of viruses recovered with magnetic beads coated with anti-HLA-DR antibody was 10- to 40-fold higher as compared to the level of viruses captured with magnetic beads coated with anti-ICAM-1, -MHC-I, -HLA-DR, or -HLA-DQ antibodies. Results from this set of experiments suggest that a greater proportion of viruses have incorporated the threshold number of host HLA-DR than of other cell surface molecules studied, which is needed to permit immunomagnetic isolation.

Of particular note are differences in the level of progeny virus captured by immunomagnetic beads coated with anti-HLA-DR antibodies. Indeed, three times more HIV-1NL43 were recovered than HIV-1LaA or HIV-1B particles with the use of anti-HLA-DR-coated magnetic particles. These data indicate that the amount of cellular HLA-DR proteins inserted on budding viruses can differ from one strain to another. Relative differences in the level of host HLA-DR proteins acquisition are not associated with a diminished ability of the antibody to react with its ligand as these different strains of HIV-1 were harvested from the same producer cell line. The strain-specificity of this phenomenon is exclusive to HLA-DR proteins as no such marked differences were seen when using magnetic beads coated with either anti-ICAM-1, -MHC-I, -HLA-DR, or -HLA-DQ antibodies.

We have next monitored whether the virus producer cell line can also act as a factor influencing the proportion of viruses harboring the threshold number of host proteins necessary to achieve selection with immunomagnetic beads. Flow cytometry analysis was first performed on studied cell lines prior to virus inoculation. Table 2 shows that CD4, MHC-I, and ICAM-1 proteins were expressed by all cell lines tested with the exception of ICAM-1 which was not detected on the surface of Molt 4 clone 8 cells. More importantly, comparison of levels of cell surface HLA-DR, -DP, and -DQ proteins, as reflected by the percentage of positive cells and the mean fluorescence intensity (indicative of the number of molecules per single cell shown on a logarithmic scale), showed that expression was high on RAJI-CD4, moderate on HUT 78, low on H9, and absent on Molt 4 clone 8 cells. Immunomagnetic recovery of virus stocks (HIV-1NL43) grown on these human CD4+ lymphoid cell lines was next carried out. Results from Fig. 2 indicate that magnetic beads coated with anti-ICAM-1, -MHC-I, -HLA-DR, and -HLA-DQ antibodies captured low to moderate amounts of viruses harvested from either RAJI-CD4 or HUT 78 cells. As expected, virus from Molt 4 clone 8 could
not be recovered with the use of magnetic beads coated with anti-ICAM-1, -HLA-DR, -DP, and -DQ antibodies. Progeny virus produced by H9 cells could not be isolated by magnetic beads coated with anti-HLA-DR and -DQ monoclonal antibodies. Interestingly, anti-HLA-DR antibodies were still the most efficient to capture HIV-1<sup>NL4-3</sup> particles independently of the producer cell line (RAJI-CD4, HUT 78, or H9 cells). Furthermore, the amount of virus particles captured with magnetic beads coated with anti-HLA-DR antibodies was six- to sevenfold higher for progeny virus generated by RAJI-CD4 than for viruses harvested from either HUT 78 or H9 cells. These results suggest that the proportion of budding virus particles with the number of host HLA-DR molecules sufficient to permit immunomagnetic isolation is also influenced by the virus producer cell line.

Virus stocks used in our experiments were all standardized according to their p24 content. It is widely accepted that virus-associated p24 may differ depending among other things, on the virally induced cytopathic effect. To evaluate whether this factor could play a role in our observations, we first evaluated the proportion of pelletable virus-associated p24 for each virus stock. Table 3 shows that the percentage of pelletable p24 was comparable for the three virus stocks harvested from RAJI-CD4. However, the percentage of virus-associated p24 was consistently lower in progeny virus originating from RAJI-CD4 (28.8 to 46.8%) than from either HUT 78 (73.9%), H9 (64.3%), and Molt 4 clone 8 cells (66.0%). These results suggest that, at least under our experimental con-
ditions, the percentage of virion-associated p24 depends more on the producer cell line than on the virus strain. This is most likely attributed to the higher virus-induced cytopathogenicity in RAJI-CD4 than in others cell lines tested (data not shown). We next determined the percentage of virus recovery when using anti-HLA-DR-coated magnetic beads after taking into account the amount of pelletable virus-associated p24. Virus recovery rate of HIV-1\textsubscript{NL4-3} (100%) was 2- to 4-fold higher than that of HIV-1\textsubscript{LAI} (45.8%) and HIV-1\textsubscript{IIIB} (27.4%), respectively, for virus stocks generated in RAJI-CD4 cells. Of interest is the finding that the totality of HIV-1\textsubscript{NL4-3} grown on RAJI-CD4 cells was recovered with immunomagnetic beads coated with anti-HLA-DR antibody. The percentage of immunomagnetically isolated HIV-1\textsubscript{NL4-3} was low and comparable when virus progeny virus were harvested from either HUT 78 (10.0%) or H9 (9.3%) cells, while it was shown to be 10-fold higher when virus particles were grown in RAJI-CD4 cells (100%).

These results demonstrate that the observed differences in the virus recovery rate by magnetic beads coated with anti-HLA-DR antibodies are independent of the percentage of pelletable p24. Furthermore, it confirmed our previous data, suggesting that incorporation of host HLA-DR molecules into budding viruses is both strain- and cell type-specific.

HLA-DR proteins are also the most abundant cell-derived membrane components on clinical isolates of HIV-1 grown on primary mitogen-stimulated mononuclear cells.

Similar studies were extended to primary isolates harvested from PHA-activated normal peripheral blood mononuclear cells to more closely parallel physiological conditions. The pattern of virally inserted cell membrane proteins in low-passage clinical strains of HIV-1 closely resembles our previous findings with laboratory isolates (Fig. 3). Indeed, we have observed that HLA-DR proteins remain the most abundant cell-derived molecules present on clinical virus isolates harvested from primary mononuclear cells, although lower levels of host molecules were acquired by primary clinical isolates as compared to laboratory strains of HIV-1.

**DISCUSSION**

Our studies focused on the incorporation within HIV-1 of host HLA-DR proteins based on our preliminary observations, suggesting that this cell surface component is the most abundant virally incorporated host protein. Another factor that urged us to concentrate our efforts on this cellular protein is that it may modulate the biology of HIV as MHC-II is the physiological counterligand of CD4 (Doyle and Strominger, 1987). To evaluate in a semi-quantitative fashion the rate of incorporation of cell membrane proteins on HIV-1, we developed a capture assay based on the use of magnetic beads coated with specific monoclonal antibodies. Beyond the fact that we confirmed the presence of CD54, MHC-I, and HLA-DR on HIV-1, results from our study also indicated that incorporation of host cellular components on newly synthesized viruses quantitatively differ depending of the viral strain and the producer cell line.

We have been unable to detect the presence of cellular HLA-DP and HLA-DQ proteins on HIV-1\textsubscript{NL4-3} grown on H9 cells, which is in agreement with previous studies using the same cell line but infected with HIV-1\textsubscript{IIIB} (Arthur et al., 1992; Henderson et al., 1987; Hoxie et al., 1987). On the other hand, such host-derived molecules were detected on HIV-1\textsubscript{NL4-3} harvested from RAJI-CD4 and HUT 78 cells. To the best of our knowledge, this is the first demonstration of the presence of cellular HLA-DP and HLA-DQ proteins on budding HIV-1. The inability to isolate progeny virus grown in H9 cells with magnetic beads coated with anti-HLA-DP and HLA-DQ antibodies is most likely attributed to insufficient amounts of virally embedded cellular HLA-DP and -DQ proteins. Perhaps this amount is under the minimal threshold number of virally acquired host proteins that is needed to allow an efficient capture by antibody-coated magnetic beads. Thus, our observations suggest that the level of surface expression would then influence the number of virally inserted host HLA-DP and HLA-DQ proteins within budding virus particles since lower levels of cell surface HLA-DP and HLA-DQ were detected on H9 cells as compared to HUT 78 cells.

More importantly, data from our comparative studies have indicated that the capacity to acquire cell membrane HLA-DR proteins is virus strain-specific as immu-
nomagnetic recovery of HIV-1NL4-3 is more efficient than capture of HIV-1LAI and HIV-1IIIEF. NL4-3 is a chimeric molecular clone that is separated in two halves by a unique EcoRI cleaving site located in vpr gene (Adachi et al., 1986). Its 5’ half is derived from the provirus NY5, while its 3’ end originates from the provirus LAI. Since statistically different immunomagnetic (anti-HLA-DR) virus recovery rates were seen between NL4-3 and LAI grown on the same cell line (RAJ1-CD4) and as both virus strains share the same 3’ half of the genome, it suggests that a genetic determinant located in the 5’ half of the HIV-1NL4-3 genome is responsible for the higher incorporation of host HLA-DR proteins. Further studies are needed to identify the viral protein(s) that directly or indirectly lead(s) to higher acquisition of cell-derived HLA-DR molecules on HIV-1. We could not detect any marked differences in the efficiency of HLA-DR molecules uptake for the three clinical virus isolates tested. We believe that more low-passage clinical strains of HIV-1 will have to be analyzed to answer this issue.

Our in vitro studies suggested that the incorporation of HLA-DR molecules is also influenced by the virus producer cell line. Two different mechanisms, which are not mutually exclusive, may be responsible for the observed phenomenon. The first possibility being that the rate of HIV-DR incorporation is highly dependent on the levels of HLA-DR expressed on the surface of the producer cell line. The second scenario being that some specific HLA-DR determinants would be more preferentially and/or efficiently incorporated within budding virions. The demonstration that comparable amounts of viruses were immunomagnetically captured when HIV-1NL4-3 was harvested from H9 and HUT 78 cells, two cell lines that express different levels of surface HLA-DR, suggests that the surface expression level on the producer cell plays a minor role in the rate of incorporation within HIV-1 of cellular constituents. The importance of the genetic background in the incorporation of host HLA-DR within budding HIV-1 particles is shown by the observation that a higher proportion of HIV-1NL4-3 progeny incorporates cellular HLA-DR proteins when the virus stock is harvested from RAJI-CD4 than from either H9 or HUT 78. It is of interest to specify that RAJI-CD4 cells carry different HLA-DR molecules (HLA-DR3 and HLA-DRw10) (Herman et al., 1990) than H9 and HUT 78 (HLA-DR4 and HLA-DRw53) (Arthur et al., 1995), which share the same genetic background as H9 is a subclone of HUT 78. These noticed changes in the immunomagnetic capture of viruses are not mediated by putative changes in the binding affinity of the anti-HLA-DR antibody used (clone 2.06) since it recognizes a monomorphic region of HLA-DR (Charbon and McDevitt, 1979). Thus, we conclude that a more selective incorporation of some specific HLA-DR determinants might occur within budding HIV-1 particles. Finally, we were able to demonstrate that the observed virus strain- and cell type-specificity of HLA-DR incorporation is not mediated by differences in the percentage of pelletable p24 found in each virus stock.

Constitutive expression of class II molecules is mainly restricted to antigen-presenting cells such as macrophages, B cells, and dendritic cells (Owen and Crumpton, 1987). In addition, normal peripheral T cells have been reported to express MHC-II proteins following antigen or alloantigen stimulation (Evans et al., 1978). Dendritic cells are in close proximity to T cells in the lymph nodes (Austyn et al., 1988; Witmer and Steinman, 1984) and these cells are known to express very high levels of MHC-II molecules and to be potent antigen-presenting cells in vitro and in vivo (Austyn and Morris, 1988; Young and Steinman, 1988). HIV-1 has been isolated from stimulated CD4+ T lymphocytes (Schnittman et al., 1989), monocytes/macrophages (Ho et al., 1986; Meltzer et al., 1990; Popovic and Gartner, 1987), and the presence of the virus has also been detected in Langerhan’s cells (dendritic cells located in the epidermis) (Rappersberger et al., 1988) and in follicular dendritic cells of lymph nodes (Spiegel et al., 1992; Tenner-Racz et al., 1985). Interestingly, a higher frequency of HIV-1-infected cells was found in lymphoid tissues, such as lymph nodes, in comparison with the frequency of infected cells in peripheral blood mononuclear cells (Pantaleo et al., 1991). Altogether these results suggest that viral particles, in infected individuals, originate primarily from cells expressing MHC-II molecules. The demonstration that low-passage clinical isolates of HIV-1 harvested from primary cultures of mononuclear cells do indeed incorporate host HLA-DR proteins further reinforces the in vivo relevance of our studies.

Recently, several studies have provided evidence for the biological functions of virus-acquired host molecules. Lee Guo and Hildreth (1995) reported that the cell adhesion molecule CD44 retains its biological activity when incorporated within budding virions. The demonstration that comparable amounts of viruses were immunomagnetically captured when HIV-1NL4-3 was harvested from H9 and HUT 78 cells, two cell lines that express different levels of surface HLA-DR, suggests that the surface expression level on the producer cell plays a minor role in the rate of incorporation within HIV-1 of cellular constituents. The importance of the genetic background in the incorporation of host HLA-DR within budding HIV-1 particles is shown by the observation that a higher proportion of HIV-1NL4-3 progeny incorporates cellular HLA-DR proteins when the virus stock is harvested from RAJI-CD4 than from either H9 or HUT 78. It is of interest to specify that RAJI-CD4 cells carry different HLA-DR molecules (HLA-DR3 and HLA-DRw10) (Herman et al., 1990) than H9 and HUT 78 (HLA-DR4 and HLA-DRw53) (Arthur et al., 1995), which share the same genetic background as H9 is a subclone of HUT 78. These noticed changes in the immunomagnetic capture of viruses are not mediated by putative changes in the binding affinity of the anti-HLA-DR antibody used (clone 2.06) since it recognizes a monomorphic region of HLA-DR (Charbon and McDevitt, 1979). Thus, we conclude that a more selective incorporation of some specific HLA-DR determinants might occur within budding HIV-1 particles. Finally, we were able to demonstrate that the observed virus strain- and cell type-specificity of HLA-DR incorporation is not mediated by differences in the percentage of pelletable p24 found in each virus stock.

Constitutive expression of class II molecules is mainly restricted to antigen-presenting cells such as macrophages, B cells, and dendritic cells (Owen and Crumpton, 1987). In addition, normal peripheral T cells have been reported to express MHC-II proteins following antigen or alloantigen stimulation (Evans et al., 1978). Dendritic cells are in close proximity to T cells in the lymph nodes (Austyn et al., 1988; Witmer and Steinman, 1984) and these cells are known to express very high levels of MHC-II molecules and to be potent antigen-presenting cells in vitro and in vivo (Austyn and Morris, 1988; Young and Steinman, 1988). HIV-1 has been isolated from stimulated CD4+ T lymphocytes (Schnittman et al., 1989), monocytes/macrophages (Ho et al., 1986; Meltzer et al., 1990; Popovic and Gartner, 1987), and the presence of the virus has also been detected in Langerhan’s cells (dendritic cells located in the epidermis) (Rappersberger et al., 1988) and in follicular dendritic cells of lymph nodes (Spiegel et al., 1992; Tenner-Racz et al., 1985). Interestingly, a higher frequency of HIV-1-infected cells was found in lymphoid tissues, such as lymph nodes, in comparison with the frequency of infected cells in peripheral blood mononuclear cells (Pantaleo et al., 1991). Altogether these results suggest that viral particles, in infected individuals, originate primarily from cells expressing MHC-II molecules. The demonstration that low-passage clinical isolates of HIV-1 harvested from primary cultures of mononuclear cells do indeed incorporate host HLA-DR proteins further reinforces the in vivo relevance of our studies.
shown that cellular proteins incorporated on viruses remain functionally active. Finally, protection against SIV infection in macaques was demonstrated to be conferred by vaccination with MHC-I class I (Chan et al., 1994) or HLA-DR proteins (Arthur et al., 1995). The authors concluded that the most probable explanation for these protections was due to anti-MHC-I or anti-HLA-DR elicited antibodies reacting with corresponding molecules on the virus surface.

The biological significance of the incorporation of host HLA-DR proteins within virus particles has to be determined. However, the incorporation of cellular HLA-DR molecules within HIV-1 coupled with the previously reported spontaneous loss of the external envelope gp120 (Gelderblom et al., 1987b) might imply modifications of viral infectivity. Such virions, which have lost part of their gp120, might be protected in vivo from their rapid elimination by neutralizing antibodies directed at the external viral envelope. In support of this notion, a study has demonstrated that incorporation of MHC-I protein (p21-microglobulin) within the human cytomegalovirus helps the virus to escape neutralizing antibodies (McKeating et al., 1987). Moreover, the binding of a virus carrying host HLA-DR proteins on its surface may lead to the formation of a complex made of MHC-II-peptide-T cell receptor that will induce either cell activation, anergy, or apoptosis of T cells. Another possibility is that incorporated cellular MHC-II molecules could trigger autoimmune reactions. The presence of anti-class II antibodies in patients infected with HIV-1 is of interest in this regard (de la Barrera et al., 1987). It is possible that the binding of virus-incorporated cellular HLA-DR proteins to its physiological ligand the CD4 molecule will result in more stronger and/or longer interactions between viruses and susceptible cells and may then favor the process of viral infection. This phenomenon would play a critical role for viruses expressing a number of gp120 proteins too low to provide the threshold binding energy necessary to overcome the electrostatic repulsion forces between negatively charged HIV-1 and target cells (Fenouillet et al., 1989). Assuming that the process of incorporation of cellular HLA-DR molecules may positively affect biological functions of budding virions, it is tempting to speculate that a higher insertion of such host constituents that would take place in infected individuals carrying appropriate HLA-DR alleles will facilitate the process of virus infection. Epidemiological studies support this assumption as a more rapid disease progression was seen in individuals carrying some specific HLA proteins. For example, an accelerated disease progression was detected in HIV-1-infected patients carrying the A1-B8-DR3 genotype (Kaslow et al., 1990). More recently, a strong association was found between HLA-DR1 and AIDS-Kaposi’s sarcoma, while HLA-DR3 was observed more frequently in AIDS patients with opportunistic infections (Klein et al., 1994).

In conclusion, our in vitro studies indicate that the acquisition of host HLA-DR molecules by budding HIV-1 is influenced by the virus strain and producer cell. This aspect of the biology of HIV needs to be further investigated as recent observations suggest that virion-bound host proteins retain their normal biological functions. A better understanding on how incorporation of host cell membrane proteins can modulate biological properties of viruses will provide insights into the possible contribution of nonviral components to the immunopathogenesis of HIV-1.

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