Rapid Communication

Envelope glycoproteins are dispensable for insertion of host HLA-DR molecules within nascent human immunodeficiency virus type 1 particles

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Abstract

HLA-DR is a host-derived protein present at the surface of HIV-1. To clarify the mechanism through which this molecule is inserted within viruses, we monitored whether the incorporation process might be influenced by the level of virus-encoded envelope (Env) glycoproteins. Wild-type virions and viruses either lacking or bearing lower levels of Env were produced in different cell types. Results from a virus capture test indicate that HLA-DR is efficiently incorporated and at comparable levels in the tested virus preparations. Therefore, Env does not play an active role in the acquisition of host HLA-DR by emerging HIV-1 particles.

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Human immunodeficiency virus type 1 (HIV-1) gag and env genes encode for polyproteins Pr55Gag and gp160, respectively. The latter is the precursor of the gp120 and gp41 glycoproteins that are forming the mature virus envelope (Env), which facilitates binding of the virus to target cells and mediates fusion and entry. During viral assembly, the matrix (MA) domain of Pr55Gag interacts with Env, a process leading to the packaging of Env glycoproteins into newly formed viral entities (Dorfman et al., 1994; Freed and Martin, 1995; Yu et al., 1992). Thereafter, virions egress from the producer cell and it is during this step that HIV-1 acquires a large collection of host cell membrane constituents. It has been reported that HIV-1 may actually benefit from such embedded surface molecules (reviewed in Tremblay et al., 1998). Yet, the mechanism underlying the incorporation process of host-derived molecules is poorly understood and efforts have to be put forward into defining this phenomenon thoroughly since it might modulate the pathogenesis caused by this human retrovirus. To work towards this end, we attempted to define whether HLA-DR acquisition is influenced by the level of Env glycoproteins based on a previous study suggesting that Env is mandatory for the efficient insertion of human leukocyte antigen (HLA) class II proteins within HIV-1 (Poon et al., 2000). Indeed, Poon and coworkers demonstrated that the presence of Env glycoproteins, and more particularly the gp41 cytoplasmic tail, is necessary to achieve incorporation of HLA class II proteins in virions produced by the human T lymphoid cell line H9 and peripheral blood mononuclear cells (PBMCs). HLA-DR, a product of major histocompatibility complex (MHC) class II genes, is a heterodimer formed by non-covalently bound α- and β-transmembrane chains prolonged by short cytoplasmic domains. This HLA determinant is a well-studied molecule in the context of incorporation of host molecules into HIV-1 (Cantin et al., 1997a, 1997b; Castilletti et al., 1995; Rossio et al., 1995).

The acquisition of HLA-DR by HIV-1 produced in 293T cells is independent of Env

Progeny viruses were initially generated in 293T cells using a well established transient transfection-and-expres-
sion system (Fortin et al., 1997, 1998; Paquette et al., 1998). Briefly, 293T cells were co-transfected with a plasmid encoding for the class II transactivator (CIITA) (Khalil et al., 2002) to drive expression of MHC class II determinants and plasmids coding for HIV-1 viruses bearing various levels of gp120 (NL4-3 backbone). The molecular clones of HIV-1 that were used in this study include wild type (WT) NL4-3 (Adachi et al., 1986), two NL4-3 matrix mutants (34VE and 30LE) (Freed and Martin, 1995, 1996), a NL4-3 plasmid deficient for both Env and Nef (Env−/Nef−) (Connor et al., 1995), and a NL4-3 vector deficient for Env only (Env−/Nef+) (Ott et al., 1999). Table 1 shows the levels of virus-associated gp120 and p24 determined by ELISA (Paquette et al., 1998) and molar ratios of gp120 to p24 calculated on the basis of their respective molecular masses (i.e., 120 and 24 kDa, respectively) as described previously (Beauséjour and Tremblay, 2004a). Based on these molar ratios, WT, 34VE, 30LE, Env−/Nef−, and Env−/Nef+ viruses displayed 100%, 21%, 4%, 0%, and 0% gp120, respectively. Viruses stocks were ultrafiltrated (Centricon Plus-20 Biomax-100 filter devices, Millipore Corporation) to eliminate free p24. Then, viruses were submitted to a virus capture assay comprising streptavidin-coated magnetic beads (Dynal Biotech Inc.) and biotinylated monoclonal antibodies (anti-HLA-DR, clone 2.06), followed by determination of p24 concentration by a homemade enzymatic test (Martin and Tremblay, 2004). Beads coated with an isotype-matched (i.e., IgG2a) irrelevant antibody were used as controls. As illustrated in Fig. 1, comparable amounts of viruses were captured by HLA-DR-tagged beads in the WT, 34VE, 30LE, and Env−/Nef− populations, thus suggesting that HLA-DR incorporation is not influenced by the degree of virus-associated Env glycoproteins. Given that Nef is influencing the budding site of HIV-1 (Zheng et al., 2001) which could in turn affect the incorporation process based on the idea that HIV-1 has been shown to egress from infected cells through specialized microdomains called lipid rafts (Chazal and Gerlier, 2003), similar experiments were also conducted with an Env-deficient vector that carries Nef.

Table 1

<table>
<thead>
<tr>
<th>Virus stocks</th>
<th>gp120 (ng/ml)</th>
<th>p24 (ng/ml)</th>
<th>gp120/p24 molar ratios (%b)</th>
</tr>
</thead>
<tbody>
<tr>
<td>WT</td>
<td>19.0 ± 0.9</td>
<td>161 ± 8</td>
<td>2.4 100</td>
</tr>
<tr>
<td>34VE</td>
<td>31 ± 1</td>
<td>1202 ± 55</td>
<td>0.5 21</td>
</tr>
<tr>
<td>30LE</td>
<td>0.50 ± 0.03</td>
<td>103 ± 5</td>
<td>0.1 4</td>
</tr>
<tr>
<td>Env−/Nef−</td>
<td>0</td>
<td>NT</td>
<td>NA</td>
</tr>
<tr>
<td>Env−/Nef+</td>
<td>0</td>
<td>NT</td>
<td>NA</td>
</tr>
</tbody>
</table>

Results are the mean ± standard deviation of triplicates and are representative of three independent experiments. NT, not tested; NA, not applicable.

a The amounts of virus-associated gp120 and p24 were determined by enzymatic assays.

b The percentage of gp120 associated to virions of the wild type stock was arbitrarily fixed to 100.

Data from the virus capture assay using samples from two separate transfection experiments confirmed that host-encoded HLA-DR molecules are acquired by HIV-1 particles devoid of Env glycoproteins (Fig. 2). Altogether, these results also indicate that Nef is not contributing to the efficient insertion of HLA-DR within mature HIV-1 particles.

Fig. 1. Incorporation of host HLA-DR in viruses produced in 293T cells. WT, 34VE, 30LE, and Env−/Nef− viruses were produced in HLA-DR-expressing 293T cells and incubated with streptavidin-coated magnetic beads tagged with biotinylated anti-HLA-DR antibodies (2.06) or isotype-matched irrelevant antibodies (IgG2a). The amounts of precipitated viruses were estimated with a p24 test. Data shown are the means ± SD of triplicate samples. Comparison of means using single-factor ANOVA and Dunnett’s test indicates that there are no statistically significant differences between levels of immunoprecipitated WT, 34VE, 30LE, or Env−/Nef− viruses by anti-HLA-DR antibodies (P < 0.05).

Fig. 2. Efficient incorporation of host HLA-DR in viruses produced in 293T cells. WT and Env−/Nef+ viruses were produced in HLA-DR-expressing 293T cells and incubated with streptavidin-coated magnetic beads tagged with biotinylated anti-HLA-DR antibodies (2.06) or isotype-matched irrelevant antibodies (IgG2a). The amounts of precipitated viruses were estimated with a p24 test. Data shown are the means ± SD of triplicate samples.
Env glycoproteins are not responsible for HLA-DR incorporation in virions produced in more natural cellular reservoirs

Although studies performed in 293T cells are informative, this human epithelial cell line might bear features distinct from the more natural cellular reservoirs of HIV-1, i.e., mononuclear cells from the immune system. Moreover, we wanted to validate our findings in the same cell types that were used to demonstrate the importance of Env packaging in HLA class II acquisition by HIV-1 (Poon et al., 2000). This goal was achieved by first producing WT and Env−/−Nef− viruses pseudotyped with the broad-host-range vesicular stomatitis virus envelope glycoprotein G (VSV-G) in 293T cells. The pseudotyping strategy with VSV-G allows bypassing the natural mode of HIV-1 entry and not only broadens the natural virus tropism but also significantly enhances virus infectivity (Luo et al., 1998). Next, pseudotyped viruses were used to infect H9 cells and PBMCs from three healthy donors that were stimulated for 72 h with PHA-L (1 μg/ml; Sigma) and recombinant human IL-2 (50 U/ml). Additionally, RAJI-CD4 cells were also infected with pseudotyped viruses because these cells express high levels of MHC class II molecules (Accolla, 1983). Viruses released from infected cells were ultrafiltrated and subjected to the virus capture test. In agreement with our findings with 293T cells, both WT and Env/Nef-deficient viruses produced by PBMCs, H9, and RAJI-CD4 cells were found to acquire host HLA-DR at comparable levels (data not shown).

Studies were also carried out in the two cellular subsets recognized as the major reservoirs of HIV-1, namely macrophages and CD4+ T lymphocytes. Autologous monocyte-derived macrophages (MDMs) and purified CD4+ T cells were obtained from three healthy donors before infection with VSV-G pseudotyped WT, 34VE, and 30LE viruses. Once again, we could not draw any conclusion on the possible involvement of Env glycoproteins in the efficient incorporation of host-derived HLA-DR into the virion. Indeed, as shown in Fig. 3, viruses that carry very low amounts of gp120 (i.e., 30LE) were captured with a comparable efficiency as WT virions when using beads coated with anti-HLA-DR antibodies. The virus producer cell type (i.e., MDMs or CD4+ T lymphocytes) has no effect on the process of HLA-DR incorporation process by WT or matrix mutant viruses. It should be noted that similar observations were made when testing progeny viruses harvested from autologous MDMs and CD4+ T cells inoculated with WT and Env−/−Nef+ viruses that were pseudotyped with VSV-G (data not shown).

In the present work, we provide evidence that host HLA-DR is found embedded within HIV-1 particles that either do not bear or carry much lower levels of Env glycoproteins compared to wild-type viruses. This observation parallels what is seen with ICAM-1 since this cell surface adhesion molecule is efficiently anchored on virions independently of Env (Beauséjour and Tremblay, 2004a), but is in sharp contrast with the study by Poon and colleagues which is focused on HLA class II incorporation (Poon et al., 2000). The explanation for this discrepancy is currently unknown, but differences in experimental methodologies may account for the different results. For example, although both studies have studied the same viral strain (i.e., NL4-3), we used a semi-quantitative immunocapture assay with a monoclonal anti-HLA-DR antibody that was followed by detection of
captured viruses by a sensitive p24 test, a technique that detects as low as 31.25 pg/ml of p24 (Martin and Tremblay, 2004), whereas Poon and co-workers first immunoprecipitated viruses through the use of a polyclonal anti-HLA class II antibody before detection by immunoblotting for Gag proteins that can detect a minimum of 1–2 ng of a specific protein (Burnette, 1981).

In summary, the precise mechanism through which host-encoded HLA-DR molecules are incorporated within budding HIV-1 particles is still mysterious and further studies are needed to shed light on this issue. We are currently defining whether the association of HLA-DR with the cytoskeleton might contribute to the efficient incorporation of this cell surface protein onto HIV-1 based on the idea that oligomerization of HLA-DR molecules induces their association with the cytoskeleton and their recruitment into lipid rafts (El Fakhry et al., 2004). Moreover, it has been recently suggested that some cytoplasmic linker proteins might be involved in acquisition of host ICAM-1 by nascent HIV-1 virions (Beauséjour and Tremblay, 2004b).

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