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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.

Keywords: Envelope glycoproteins; HLA-DR; Human immunodeficiency virus type 1

Human immunodeficiency virus type 1 (HIV-1) *gag* and *env* genes encode for polyproteins $Pr55^{Gag}$ 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 $Pr55^{Gag}$ 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-

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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. Virus 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., IgG_{2a}) 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
gp120 associated to wild type and mutated HIV-1 virions

Virus stocks	gp120 (ng/ml)[a]	p24 (ng/ml)[a]	gp120/p24 molar ratios (10^{-2})	gp120/p24 (%)[b]
WT	19.0 ± 0.9	161 ± 8	2.4	100
34VE	31 ± 1	1202 ± 55	0.5	21
30LE	0.50 ± 0.03	103 ± 5	0.1	4
Env−/Nef−	0	NT	NA	NA
Env−/Nef+	0	NT	NA	NA

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.

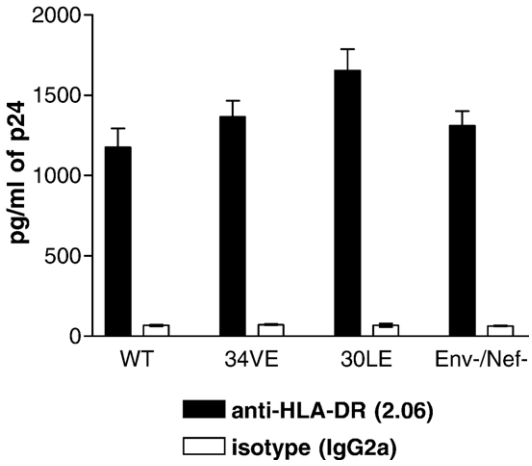

Fig. 1. Incorporation of host HLA-DR in viruses produced in 293T cells. WT, 34VE, 30LE, and Env−/Nef− viruses were produced in HLA-DR-expressing 293T cells and incubated with streptavidin-coated magnetic beads tagged with biotinylated anti-HLA-DR antibodies (2.06) or isotype-matched irrelevant antibodies (IgG_{2a}). The amounts of precipitated viruses were estimated with a p24 test. Data shown are the means ± SD of triplicate samples and are representative of three independent experiments. 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 ($P < 0.05$).

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.

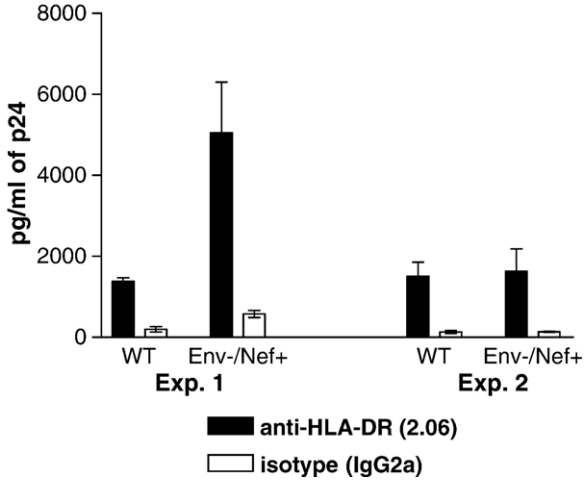

Fig. 2. Efficient incorporation of host HLA-DR in viruses produced in 293T cells. WT and Env−/Nef+ viruses were produced in HLA-DR-expressing 293T cells and incubated with streptavidin-coated magnetic beads tagged with biotinylated anti-HLA-DR antibodies (2.06) or isotype-matched irrelevant antibodies (IgG_{2a}). The amounts of precipitated viruses were estimated with a p24 test. Data shown are the means ± SD of triplicate samples.