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Envelope glycoproteins sampling states 2/3 are susceptible to ADCC by sera from HIV-1-infected individuals

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ABSTRACT

Recent analysis of HIV-1 envelope glycoproteins (Env) dynamics showed that the unliganded Env trimer can potentially sample three conformations: a metastable "closed" conformation (State 1), an "open" CD4-bound conformation (State 3), and an intermediate "partially open" conformation (State 2). HIV-1 evolved several mechanisms to avoid "opening" its Env in order to evade immune responses such as antibody-dependent cellular cytotoxicity (ADCC), which preferentially targets Envs in the CD4-bound conformation on the surface of infected cells. Here we took advantage of a well-characterized single-residue change in the gp120 trimer association domain to modify Env conformation and evaluate its impact on ADCC responses. We found that cells infected with viruses expressing Env stabilized in States 2/3 become highly susceptible to ADCC responses by sera from HIV-1-infected individuals. Our results indicate that the conformations spontaneously sampled by the Env trimer at the surface of infected cells has a significant impact on ADCC responses.

1. Introduction

The entry of human immunodeficiency virus (HIV-1) into the host cell is mediated by the viral envelope glycoproteins (Envs), which are derived by proteolytic cleavage of a trimeric gp160 Env precursor (Allan et al., 1985; Robey et al., 1985; Wyatt and Sodroski, 1998). The mature Env complex is composed of three gp120 surface subunits and three gp41 transmembrane subunits. Env is a metastable molecule which transits from its unliganded "closed" high energy conformation (State 1) to an "open" CD4-bound low energy conformation (State 3). CD4 engagement drives Env into an intermediate "partially open" conformation and then into State 3, a prehairpin intermediate conformation (Herschhorn et al., 2016; Munro et al., 2014). CCR5 or CXCR4 coreceptor interaction with the gp120 promotes additional conformational changes in gp41 resulting in the formation of a six-helix bundle formed by HR1 and HR2 heptad repeats resulting in the fusion of viral and cellular membranes (Chan et al., 1997; Lu et al., 1995; Weissenhorn et al., 1997).

Env represents the only virus-specific antigen exposed at the surface of infected cells and thus is a major target for antibody-mediated immune responses, including antibody-dependent cellular cytotoxicity (ADCC). The unliganded Env of most primary HIV-1 isolates assumes a "closed" State 1 conformation (Julien et al., 2013; Liu et al., 2008; Lyumkis et al., 2013; Mao et al., 2012; Munro et al., 2014; Pancera et al., 2014; White et al., 2010), which renders the trimer relatively resistant to antibody attack. Env interaction with CD4 (Veillette et al., 2015, 2014b), large alterations in the Phe 43 cavity (Prevost et al., 2017) and small CD4-mimetics (CD4mc) (Richard et al., 2016a, 2015) have been shown to trigger Env to sample downstream conformations and render HIV-1-infected cells susceptible to ADCC responses. Thus, downstream conformations from State 1 appear to be preferentially recognized by ADCC-mediating antibodies that are present in the sera of HIV-1-infected individuals (Veillette et al., 2015).

In order to limit the recognition of Env at the surface of infected cells, HIV-1 has evolved sophisticated mechanisms to efficiently internalize Env (von Bredow et al., 2015), to counteract the host restriction factor BST-2 with the viral Vpu protein (Alvarez et al., 2014; Arias et al., 2014; Veillette et al., 2014b), and to downregulate CD4 using Nef and Vpu (Veillette et al., 2015, 2014b). Moreover, multiple intermolecular interactions within the Env trimer contribute to the

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maintenance of this relatively "antibody-resistant" State 1 conformation, including the gp120 β20-β21 element and the V1V2 and V3 variable loops (Herschhorn et al., 2017, 2016; Kwon et al., 2012). For example, mutation of "restraining" residues within V1V2 were shown to enable Env to spontaneously sample lower energy State 2 and 3 conformations. These Env variants were reported to be more susceptible to neutralization by State 2/3-preferring ligands such as soluble CD4 (sCD4), small CD4-mimetics (CD4mc) and CD4-induced (CD4i) antibodies (Herschhorn et al., 2016). However, their impact on ADCC responses remains unknown. Here we tested the influence of a V2 State 2/3-stabilizing Env mutation, L193A, on ADCC responses mediated by sera from HIV-1-infected individuals.

2. Materials and methods

2.1. Cell lines and isolation of primary cells

293T human embryonic kidney (obtained from ATCC, Cat# CRL-3216, RRID: CVCL 0063), and primary CD4+ T cells were grown as previously described (Richard et al., 2010; Veillette et al., 2014b). CD4+ T lymphocytes were purified from resting PBMCs by negative selection and activated as previously described (Richard et al., 2015). Research adhered to the ethical guidelines of CRCHUM and was reviewed and approved by the CRCHUM institutional review board (ethics committee). Research adhered to the standards indicated by the Declaration of Helsinki.

2.2. Viral production and infections

In order to achieve the same level of infection among the different mutants tested, vesicular stomatitis virus G (VSVG)-pseudotyped HIV-1 viruses were produced and titrated as described (Veillette et al., 2015). Viruses were used to infect primary CD4+ T cells from healthy HIV-negative donors by spin infection at 800 g for 1 h in 96-well plates at 25 °C. Under these conditions we obtained equivalent levels of infection for all viruses tested.

2.3. Antibodies and sera

The following antibodies were used to assess Env cell-surface staining: conformation-independent anti-gp120 outer-domain 2G12 (NIH AIDS Reagent Program), State 1-preferring anti-gp120 antibodies VRC03 (NIH AIDS Reagent Program), PGT151 (IAVI) and PG9 (Polymun), State 2/3-preferring anti-gp120 antibodies 17b, 19b, A32, F105 and anti-gp41 antibodies 7B2 and F240 (NIH AIDS Reagent Program). The monoclonal antibody anti-CD4 OKT4 (BioLegend) binds to the D3 domain of CD4 and was used to measure cell-surface levels of CD4, as described (Veillette et al., 2014b). Secondary antibodies goat anti-mouse and anti-human coupled to Alexa Fluor 647 (Invitrogen) were used in flow cytometry experiments. Sera from HIV-infected and healthy donors were collected, heat-inactivated and conserved at −80 °C until use. Written Informed consent was obtained from all study participants (the Montreal Primary HIV Infection Cohort (Fontaine et al., 2011, 2009) and the Canadian Cohort of HIV Infected Slow Progressors (International et al., 2010; Kamya et al., 2011; Peretz et al., 2007)). A random number generator (GraphPad, QuickCalcs) was used to randomly select a number of sera for each experiment.

2.4. Plasmids

The plasmid encoding the HIV-1 transmitted founder (TF) infectious molecular clone (IMC) CH58 and CH77 were previously described (Bar et al., 2012; Fenton-May et al., 2013; Ochsenbauer et al., 2012; Parrish et al., 2013; Richard et al., 2015). Individual Env mutations were introduced using the QuikChange II XL site-directed mutagenesis protocol (Stratagene) and verified by sequencing.

2.5. Flow cytometry analysis of cell-surface staining

Using the standard calcium phosphate method, 7 μg of each IMC was transfected into 2×10^6 293T cells. At 48 h post transfection, 293 T cells were stained with anti-Env antibodies (5 μg/mL) followed by intracellular p24 staining (PE-anti-p24, clone KC57; Beckman Coulter/Immunotech, Hialeah, FL; 1:100 final concentration), to identify transfected cells. Alternatively, to evaluate soluble CD4 (sCD4) binding to the different Envs expressed at the cell surface, transfected 293 T cells were incubated with sCD4 (5 μg/mL), followed with a staining by the monoclonal anti-CD4 OKT4 antibody.

Cell-surface staining of primary CD4+ T cells was performed as previously described (Richard et al., 2015; Veillette et al., 2015). MFI histograms show signal on live infected populations. Binding of HIV-1-infected cells by HIV+ sera (1:1000 dilution) or anti-Env mAbs (5 μg/mL) were performed 48 h after infection. The percentage of infected cells (p24+ cells) was determined by gating the living cell population based on viability dye staining (Aqua Vivid, Thermo Fisher Scientific, Cat# L43957). Samples were analyzed on an LSRII cytometer (BD Biosciences, Mississauga, ON, Canada) and data analysis was performed using FlowJo v10.0.7 (Tree Star, Ashland, OR, USA).

2.6. ADCC responses

Measurement of ADCC-mediated killing was performed with a previously described assay (Richard et al., 2015). Primary CD4+ T cells isolated from five healthy HIV negative donors were infected for 48 h with the different IMCs. In order to avoid the potential bias induced by the presence of gp120-coated uninfected bystander CD4+ T cells in ADCC measurements (Richard et al., 2016b), $CD4^{high}$ T cells were removed from the target cell population using Dynabeads® $CD4^+$ positive selection kit (Invitrogen) at a ratio of 25 μl of beads per million cells. Of note, these beads do not distinguish between p24+ and p24- cells. Their capacity to enrich $CD4^{low}$ cells is based in their ability to select for $CD4^{high}$ cells. Negative selection of $CD4^{low}$ T cells was assessed by double staining with anti-CD4 OKT4 mAb and anti-p24 mAb (KC57, Beckman Coulter). Productively-infected primary CD4 T cells ($CD4^{low}$, p24+) were incubated with autologous PBMC in presence of HIV+ or HIV- sera (1:1000) for 5 h at 37 °C. Cytometry beads were used to normalize the number of productively-infected cells left after the ADCC reaction, as described (Richard et al., 2014, 2016b). The percentage of cytotoxicity (% ADCC) was calculated with the following formula: (Normalized number of Targets plus Effectors) - (Normalized number of Targets plus Effectors plus serum)/(Normalized number of Targets) by gating infected lived target cells.

2.7. Immunoprecipitation of envelope glycoproteins

3×10^5 293T cells were transfected by the calcium phosphate method with the different IMCs. One day after transfection, cells were metabolically labeled for 16 h with 100 μCi/mL [^{35}S]methionine-cysteine ([^{35}S] Protein Labeling Mix; Perkin-Elmer) in Dulbecco's modified Eagle's medium lacking methionine and cysteine and supplemented with 5% dialyzed fetal bovine serum. Cells were subsequently lysed in RIPA buffer (140 mM NaCl, 8 mM Na_2HPO_4, 2 mM NaH_2PO_4, 1% NP40, 0.05% sodium dodecyl sulfate (SDS)). Precipitation of radiolabeled envelope glycoproteins from cell lysates or medium was performed with a mixture of sera from HIV-1-infected individuals in the presence of 50 μl of 10% Protein A-Sepharose (American BioSciences) at 4 °C. The association index is a measure of the ability of the mutant gp120 molecule to remain associated with the Env trimer complex on the expressing cell, relative to that of the wild-type Env trimers. The association index is calculated as follows: association index = ([mutant $gp120]_{cell}$ × [wild-type $gp120]_{supernatant}$)/ ([mutant $gp120]_{supernatant}$ × [wild-type $gp120]_{cell}$).