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Research Paper

Small CD4 Mimetics Prevent HIV-1 Uninfected Bystander CD4 + T Cell Killing Mediated by Antibody-dependent Cell-mediated Cytotoxicity

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ABSTRACT

Human immunodeficiency virus type 1 (HIV-1) infection causes a progressive depletion of CD4 + T cells. Despite its importance for HIV-1 pathogenesis, the precise mechanisms underlying CD4 + T-cell depletion remain incompletely understood. Here we make the surprising observation that antibody-dependent cell-mediated cytotoxicity (ADCC) mediates the death of uninfected bystander CD4 + T cells in cultures of HIV-1-infected cells. While HIV-1-infected cells are protected from ADCC by the action of the viral Vpu and Nef proteins, uninfected bystander CD4 + T cells bind gp120 shed from productively infected cells and are efficiently recognized by ADCC-mediating antibodies. Thus, gp120 shedding represents a viral mechanism to divert ADCC responses towards uninfected bystander CD4 + T cells. Importantly, CD4-mimetic molecules redirect ADCC responses from uninfected bystander cells to HIV-1-infected cells; therefore, CD4-mimetic compounds might have therapeutic utility in new strategies aimed at specifically eliminating HIV-1-infected cells.

1. Introduction

Human immunodeficiency virus (HIV) infection causes the progressive loss of CD4 + T cells, which leads to acquired immunodeficiency syndrome (AIDS) and death. Despite its clinical importance, the precise mechanism(s) underlying CD4 + T cell depletion during HIV-1 infection remains poorly understood (Thomas, 2009; Grossman et al., 2002; Mccune, 2001). HIV replication is known to directly induce the death of infected CD4 + T cells (Hazenberg et al., 2000; Cummins and Badley, 2014) by a plethora of mechanisms, including envelope glycoprotein-mediated cytotoxicity, apoptosis via activation of Caspases and DNA damage responses (Sodroski et al., 1986; Lifson et al., 1986; Cao et al., 1996; Labonte et al., 2003; Cooper et al., 2013; Sainski et al., 2011). The massive depletion of CD4 + T cells in SHIV-infected monkeys has been shown to depend, at least in part, on the membrane-fusing capacity of the viral envelope glycoproteins (Etemad-Moghadam et al., 2001). In addition, HIV-specific CD8 + cytotoxic T cells also play a major role in eliminating HIV-infected cells (Mcmichael and Rowland-Jones, 2001). However, the mechanisms of uninfected bystander CD4 + T cell death and the contribution of this process to HIV-1 pathogenesis are not well understood. It has been described that uninfected CD4 + T cells die from apoptosis induced by over-expression of several death ligands (Cummins and Badley, 2014), activation-induced cell-death caused by a state of hyper-inflammation (Alimonti et al., 2003), direct cytotoxicity mediated by some HIV proteins including Tat, Vpr, Nef and gp120 (Varbanov et al., 2006), and caspase-1-dependent

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pyroptosis driven by abortive infections (Cummins and Badley, 2014; Doitsh et al., 2014; Monroe et al., 2014). In fact, killing of uninfected CD4 + T cells was estimated to account for the high rate of turnover of CD4 + T cells in HIV-infected individuals (Mccune et al., 2000; Mohri et al., 2001; Meyaard et al., 1992; Matrajt et al., 2014). However, no particular mechanism has been identified that could account for this short half-life.

The HIV-1 envelope glycoprotein (Env) trimer is derived from proteolytic cleavage of a trimeric gp160 precursor (Allan et al., 1985; Robey et al., 1985) and is composed of gp120 exterior and gp41 transmembrane subunits. The gp120 exterior subunit is retained on the trimer via labile, non-covalent interactions with the gp41 ectodomain. This results in spontaneous dissociation of gp120 from gp41, known as gp120 shedding (Helseth et al., 1991; Yang et al., 2003; Finzi et al., 2010). Accordingly, significant levels of soluble gp120 were found in blood and tissues of HIV-infected individuals (Santosuosso et al., 2009; Oh et al., 1992; Rychert et al., 2010). Interestingly, it has been suggested that shed gp120 bound to the surface of lymphocytes could serve as an effective target for immune destruction by patient antibodies and effector cells and that this could contribute to lymphocyte destruction in HIV-1-infected individuals (Lyerly et al., 1987). During HIV entry, the gp120 glycoprotein is responsible for interactions with the initial receptor, CD4 (Dalgleish et al., 1984; Klatzmann et al., 1984). CD4 induces conformational changes in Env that expose the binding site for the chemokine co-receptors (CCR5 or CXCR4) (Alkhatib et al., 1996; Choe et al., 1996; Deng et al., 1996; Dragic et al., 1996; Feng et al., 1996). We recently reported that gp120–CD4 interaction in an infected cell also results in exposure of antibody-dependent cell-mediated cytotoxicity (ADCC) epitopes recognized by antibodies present in sera, cervicovaginal fluids and breast milk from HIV-infected individuals. Interestingly, recognition of gp120–CD4 complexes at the surface of infected (Richard et al., 2015; Veillette et al., 2014b, 2015a) or gp120-coated (Batraville et al., 2014; Richard et al., 2014) cells results in ADCC-mediated killing.

Here we investigated whether antibody recognition of shed gp120 bound to the surface CD4 of bystander cells results in ADCC-mediated killing. To this end, we developed a FACS-based assay to measure the relative killing of infected CD4 + T cells versus their uninfected counterparts by ADCC-mediating antibodies and HIV + sera. We observed that HIV-1-infected cells were protected from ADCC, whereas bystander cells were highly sensitive to killing mediated by CD4-induced (CD4i) antibodies and HIV + sera. This difference in susceptibility to ADCC was due to differences in Env conformation in the two contexts. In infected cells, trimeric Env remained in its unbound conformation (i.e., shielding ADCC-mediating epitopes) due to the action of the HIV-1 Vpu and Nef proteins, which limit the surface expression of Env–CD4 complexes (Richard et al., 2015; Veillette et al., 2014b, 2015a, 2016). By contrast, in bystander CD4 + T cells, shed gp120 was bound to CD4 on the cell surface, resulting in a more open Env conformation that exposed ADCC-mediating epitopes. This raises the intriguing possibility that gp120 shedding may represent a viral mechanism to divert cytotoxic immune responses to uninfected cells. Importantly, we demonstrate here that small CD4-mimetic compounds (CD4mc) protect bystander cells by blocking gp120 binding to uninfected CD4 + T cells, while sensitizing HIV-infected cells to ADCC-mediated killing.

2. Materials and Methods

2.1. Cell Lines and Isolation of Primary Cells

293T human embryonic kidney (obtained from ATCC), CEM.NKr cells (obtained from Dr. David Evans, Harvard Medical School) and primary 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).

2.2. Viral Production, Infections, Ex Vivo Amplification and Detection of Infected Cells

Vesicular stomatitis viruses G (VSVG)-pseudotyped viruses allowed equivalent levels of infection for the different viruses used, including the D368R Env variant, and were produced and titrated as previously described (Veillette et al., 2015a). Viruses were then used to infect CEM.NKr cells or primary CD4 + T cells from healthy donors by spin infection at 800 g for 1 h in 96-well plates at 25 °C. In order to expand endogenously-infected CD4 + T cells, primary CD4 + T cells were isolated from PBMCs obtained from viremic HIV-1-infected individuals. Purified CD4 + T cells were activated with PHA-L at 10 μg/ml for 36 h and then cultured for 6 to 8 days in RPMI-1640 complete medium supplemented with rIL-2 (100 U/ml).

Detection of GFP + or p24 + infected cells was performed as previously described (Richard et al., 2015). Cells infected with the SHIV-CH505 virus were stained intracellularly for SIVmac p27 using the Cytofix/Cytoperm Fixation/Permeabilization Kit, followed by a 2F12 anti-p27 mAb primary staining (10 μg/ml) and a goat anti-mouse Alexa Fluor 647 secondary antibody staining (1:250 dilution). The percentage of infected cells (GFP +, p24 + or p27 + cells) was determined by gating the living cell population based on the viability dye staining (Aqua Vivid, Invitrogen).

2.3. Antibodies and Sera

The following Abs were used as first Ab for cell-surface staining: 1 μg/ml mouse anti-CD4 mAb OKT4 (14-0048-82; eBiosciences), 5 μg/ml human anti-HIV-1 Env mAbs 2G12 (AB002; Polymun), PGT-151 (kindly provided by P. Poignard, The Scripps Research Institute, La Jolla, CA), A32, C11, 7B2 (kindly provided by J. Robinson, Tulane University, New Orleans), F240, M785-U1 and N10-U1 (kindly provided by G. K. Lewis, University of Maryland), whereas 1 μg/ml either goat anti-mouse Alexa Fluor-594, goat anti-human Alexa Fluor 647 or goat anti-human Alexa Fluor 594 mAbs (Invitrogen) was used as a secondary Ab, and AquaVivid (Invitrogen) was used as a viability dye. The anti-SIVmac p27 antibody was purified from SIVmac p27 Hybridoma (55-2F12, NIH AIDS Reagent) (Higgins et al., 1992) using Protein A-Sepharose beads (GE, USA) according to the manufacturer's protocol.

Sera from HIV-infected and healthy donors were collected, heat-inactivated and conserved as previously described. Written informed consent was obtained from all study participants [the Montreal Primary HIV Infection Cohort (Fontaine et al., 2009, 2011) and the Canadian Cohort of HIV Infected Slow Progressors (Peretz et al., 2007; Kamya et al., 2011; International et al., 2010)], and research adhered to the ethical guidelines of CRCHUM and was reviewed and approved by the CRCHUM institutional review board (ethics committee). All sera were heat-inactivated for 30 min at 56 °C and stored at 4 °C until ready to use in subsequent experiments. A random number generator (GraphPad, QuickCalcs) was used to randomly select a number of sera for each experiment.

2.4. Plasmids and Site-directed Mutagenesis

pNL43-ADA(Env)-GFP.IRES.Nef proviral vectors (*wt* or expressing the D368R Env variant) and the VSV G-encoding plasmid (pSVCMV-IN-VSV-G) were previously described (Veillette et al., 2015a). The pNL43-GFP.IRES.Nef provirus encoding the NL43 X4-tropic Env was generated by inserting the NL43 Env into pNL43-ADA(Env)-GFP.IRES.Nef using SalI and BamHI restrictions sites, as previously described (Veillette et al., 2014b). The plasmid encoding the HIV-1 transmitted founder (T/F) IMC CH77 was previously described (Ochsenbauer et al., 2012; Bar et al., 2012; Parrish et al., 2013; Fenton-May et al., 2013; Richard et al., 2015).