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Dynamic appearance of antigenic epitopes effective for viral neutralization during membrane fusion initiated by interactions between HIV-1 envelope proteins and CD4/CXCR4☆

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ABSTRACT

HIV-1 entry into cells is mediated by interactions between the envelope (Env) gp120 and gp41 proteins with CD4 and chemokine receptors via an intermediate called the viral fusion complex (vFC). Here, mAbs were used to find the dynamic changes in expression of antigenic epitopes during vFC formation. A CD4-specific mAb (R275) and anti-vFC mAbs, designated F12-1, F13-6 and F18-4 that recognize the epitopes only appeared by the co-culture of env-transfected 293FT and CD4-transfected 293 cells, were developed by immunizing *ganp*-gene transgenic mice with an vFC-like structure formed by the same co-culture. The epitopes recognized by the mAbs appeared at different time points during vFC formation: F18-4 appeared first, followed by F13-6, and finally F12-1. The anti-vFC mAbs had little effect on vFC formation or virus neutralization; however, interestingly F12-1 and F18-4 increased exposure of the OKT4-epitope on the domain 3 in the extracellular region of CD4. R275, which recognizes the epitope closely associated with the OKT4-determinant on the domain 3, showed the marked inhibition of vFC formation and viral neutralization activity. The Ab binding to the epitopes appeared during viral membrane fusion might reinforce the appearance of the target epitopes for effective neutralization activity.

Introduction

HIV-1 infection occurs through attachment of the gp160 (gp120 and gp41 complex) trimeric envelope (Env) protein with CD4 and CXCR4/CCR5 molecules expressed on human T cells (Berger et al. 1999; Sattentau and Weiss 1988). The process of viral infection involves dynamic changes in the structure of this Env trimer (Doms and Moore 2000; Eckert and Kim 2001). Virus attachment is initiated by the binding of gp120 to CD4, which causes changes in the gp120/gp41 trimer structure, pushing gp41 towards the target cells. This creates the viral fusion complex (vFC), which interacts with the cell membrane and promotes viral entry by inserting the viral peptide sequence into CD4 T cells (Chan and Kim 1998; Sattentau et al. 1993).

Human CD4, the primary receptor for HIV-1, has four domains (D1, D2, D3, and D4) in its extracellular region (Ryu et al. 1990; Wang et al. 1990; Wu et al. 1997). The primary binding site for HIV-1 comprises residues 40–60 within the D1 domain (Arthos et al. 1989). Earlier studies using mutant CD4 molecules identified residue 87 within D2 domain as the most crucial region for cell fusion (Camerini and Seed 1990). The D2 domain forms a loop structure in close proximity to the primary gp120 binding site. Extensive studies of CD4 interactions using mAbs against the Env protein show that various epitopes interact with CD4 and may serve as targets of HIV-1 neutralization (Burton et al. 1994; Eda et al. 2006a,b; Muster et al. 1993; Trkola et al. 1995; Zolla-Pazner et al. 1995). Initial binding to CD4 occurs via all components of the vFC: the V3-epitope of gp120, the CD4 binding site (CD4bs), and the

Abbreviations: Env, envelope; RT, room temperature; RL, renilla luciferase; vFC, viral fusion complex; Tg, transgenic; spGFP, split GFP; PH, pleckstrin homology.

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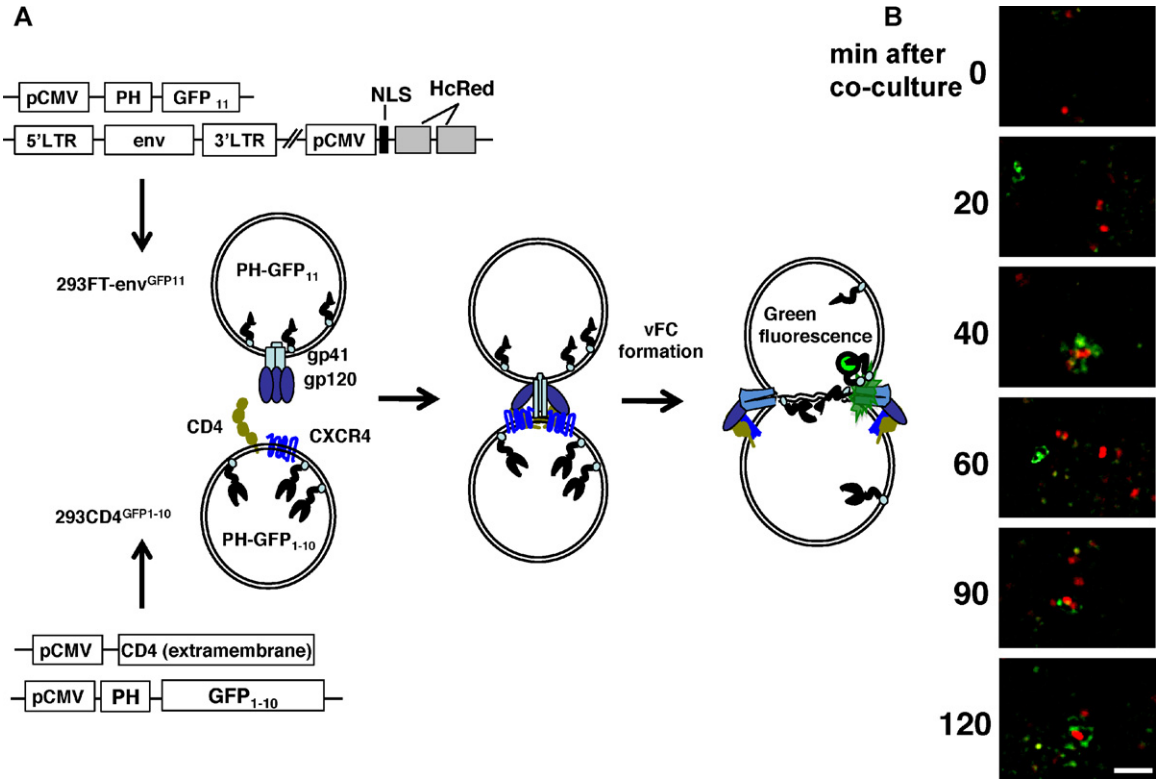

Fig. 1. A model system for monitoring the formation of vFC using spGFP. (A) 293FT-env^{GFP11} cells were prepared by transfecting 293FT cells with vectors for Env expression (driven by LTR promoter elements in conjunction with the tandem oriented HcRed protein and the nuclear localization signal (NLS) sequence required to localize the HcRed protein to the nucleus) and a fragment of GFP (GFP_{11}) linked to PH. 293CD4$^{GFP1-10}$ was prepared by transfecting pCMV-human CD4 (extra-membrane region) and pCMV-PH-GFP_{1-10} into 293 cells expressing CXCR4. The schematic diagram shows virus/membrane fusion mediated via the interaction between Env and CD4/chemokine receptors during the viral entry process. (B) Fusion of the transfectants resulted in the expression of the HcRed signal in the nuclei and the appearance of the GFP signal at the membrane. Bars = 50 μm.

gp41 transmembrane proximal epitope. Many mAbs disrupt these interactions and have a substantial neutralizing effect upon HIV-1 infection.

Further studies identified a so-called "hidden epitope" on the membrane of CD4$^+$ T cells, which is induced by gp120-CD4 binding (Thali et al. 1993; Wyatt et al. 1995; Xiang et al. 2002), and is recognized by mAbs (possessing broadly neutralizing activity) that inhibit infection by various HIV-1 virus clades (Choe et al. 2003; Decker et al. 2005; Labrijn et al. 2003). Recent studies demonstrate the feasibility of mAbs directed against vFC as neutralizing agents that prevent HIV-1 infection (Finnegan et al. 2001; Haim et al. 2007). Epitopes may be detected by mAbs produced by memory B cells isolated from the peripheral blood lymphocytes of patients showing long-term survival after HIV-1 infection (Diskin et al. 2010).

To further identify target epitopes that appear or alter during the process of viral attachment and entry into cells, we prepared vFCs formed between Env- and CD4/CXCR4-transfectants after optimization of the culture conditions by using a split Green Fluorescent Protein (spGFP) system targeted to the plasma membranes (Wang et al. 2009). The complex of the co-culture containing vFC was immunized to *ganp*-transgenic (GANP^{Tg}) mice (Sakaguchi et al. 2005), which can generate extraordinarily high-affinity mAbs against various kinds of Ags (Ono et al. 2009; Sakaguchi et al. 2009). GANP is a nuclear protein that is upregulated in germinal center B cells after immunization with T cell-dependent Ags (Sakaguchi et al. 2011). Interactions between activation-induced cytidine deaminase (AID) and GANP induce the nuclear localization of cytidine deaminase, increasing its access to *IgV*-region genes, a process necessary for the generation of high-affinity Abs (Maeda et al. 2010). In this study, we obtained various kinds of mAbs that recognize vFC, and investigated the changes in appearance of antigenic epitopes during the vFC and the effectiveness of mAbs directed at these epitopes in preventing vFC formation and viral infection *in vitro*.

Materials and methods

Cells

The 293 cells and 293CD4 cells (293 cells constitutively expressing human CD4) (Miyauchi et al., 2005) were maintained separately in Dulbecco's Modified Eagle Medium (DMEM; Invitrogen, Carlsbad, CA) with 10% (v/v) heat-inactivated fetal bovine serum (FBS; Sigma Aldrich, St. Louis, MO) at 37 °C with 5% CO_2 in a humidified incubator.

Monitoring membrane fusion with a Split Protein reporter

The engineered spGFP (composed of GFP^{1-10} and GFP^{11}) was used in the cell–cell fusion system to enable real-time monitoring of membrane fusion as previously described (Wang et al., 2009; Kondo et al. 2010). 293FT cells (Invitrogen) and 293CD4$^{GFP1-10}$ cells expressing endogenous CXCR4 (Wang et al., 2009) were maintained in G418 (500 μg/ml; Nacalai Tesque, Kyoto, Japan). 293FT cells were transfected transiently with the Env-expressing vector (pNHcRedEluc HXB2 Env) and the pdPH-GFP^{11} expression vector (Wang et al. 2009) to generate 293FT-env^{GFP11} cells.

293CD4$^{GFP1-10}$ cells (5×10^4) were layered over 293FT-env^{GFP11} cells (5×10^4, prepared 35 h prior to co-culture) in Lab-Tek® II Chamber Slides (Nalge Nunc International, Rochester, NY) at 37 °C