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Escherichia coli surface display of single-chain antibody VRC01 against HIV-1 infection

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ABSTRACT

Human immunodeficiency virus type 1 (HIV-1) transmission and infection occur mainly via the mucosal surfaces. The commensal bacteria residing in these surfaces can potentially be employed as a vehicle for delivering inhibitors to prevent HIV-1 infection. In this study, we have employed a bacteria-based strategy to display a broadly neutralizing antibody VRC01, which could potentially be used to prevent HIV-1 infection. The VRC01 antibody mimics CD4-binding to gp120 and has broadly neutralization activities against HIV-1. We have designed a construct that can express the fusion peptide of the scFv-VRC01 antibody together with the autotransporter β-barrel domain of IgAP gene from *Neisseria gonorrhoeae,* which enabled surface display of the antibody molecule. Our results indicate that the scFv-VRC01 antibody molecule was displayed on the surface of the bacteria as demonstrated by flow cytometry and immunofluorescence microscopy. The engineered bacteria can capture HIV-1 particles via surface-binding and inhibit HIV-1 infection in cell culture.

Introduction

The acquired immunodeficiency syndrome (AIDS) pandemic caused by the human immunodeficiency virus type 1 (HIV-1) has been ongoing for over three decades (Barre-Sinoussi et al., 1983; Schupbach et al., 1984). It is estimated that 39 million people have already died of AIDS-related diseases; 35 million people are living with HIV-1, and 2.1 million people are newly infected each year (UNAIDS, 2014). In spite of the recent optimism regarding a functional cure for HIV-1, there are still substantial obstacles for achieving a sterilizing cure with the current available treatment regimens. Therefore, preventing new infection is still the priority for curbing the AIDS epidemic. So far, much effort has been dedicated towards the development of an effective AIDS vaccine, but none has yet been successful (Burton et al., 2012; Johnston and Fauci, 2011; Lifson and Haigwood, 2012; O'Connell et al., 2012; Xiang, 2013). Therefore, other alternative prevention approaches are needed in order to curtail HIV-1 transmission and infection.

There are several reports on a new prevention approach using gram-positive bacteria, such as lactobacilli, for surface display of CD4 (2-domains) (Liu et al., 2008), HIV-1 entry inhibitor cyanovirin-N (CV-N) (Lagenaur et al., 2011; Liu et al., 2006; Yamamoto et al., 2013), or for secretion of a CCR5 antagonist RANTES (Vangelista et al., 2010) and HIV-1 fusion peptide inhibitors (Pusch et al., 2006) to prevent HIV-1 infection. These approaches involve the use of bacteria to synthesize anti-HIV-1 inhibitors that can be secreted or displayed on the cell surface so that the inhibitors can bind to the virus and prevent infection. Since these commensal bacteria inhabit the mucosal sites which are the port of entry for HIV-1, these protein-based inhibitors can be produced by the bacteria which can be maintained as part of the normal mucosal bacterial flora, this approach could thus be employed as a potential strategy to prevent HIV-1 infection.

Gram-negative bacteria have also been used for a number of therapeutic applications including gene delivery into the gut (Castagliuolo et al., 2005). Successful attempts include surface display of lipase (Lee et al., 2013), antimicrobial peptides (Shin et al., 2013), β-glucuronidase (Cheng et al., 2013) and also for the secretion of single-chain or Fab antibody fragments (Cheung et al., 1992; Fallecker et al., 2013; Fernandez et al., 2000). However, very few studies have been reported in the use of gram-negative bacteria as potential agents for the delivery (Abdel-Mohsen et al., 2013) of anti-HIV regimens. So far only one study has been reported in which *Escherichia coli* Nissle 1917 was used to secrete an anti-HIV-1 fusion peptide inhibitor to target gp41 glycoprotein of the virus.

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The fusion peptides from bacterial secretion can inhibit viral infection in cell culture experiments, and bacterial colonization in mice can last for weeks, or in some cases even months, however, the viral challenge experiment *in vivo* has not yet been carried out as the mice cannot directly be used for HIV-1 infection (Rao et al., 2005). Surface display of anti-HIV-1 inhibitors on gram-negative bacteria is another approach in this commensal bacterial strategy, but it has not yet been tested. For surface display, the bacterial transporter genes must be used to translocate the molecules of interest onto the cell surface (Castagliuolo et al., 2005; Fairman et al., 2011; Jose et al., 2012). Among the known transporters, the autotranspoter (AT) is one of the most studied, and its structure and translocating mechanisms have been reported recently (Benz and Schmidt, 2011; Ieva and Bernstein, 2009; Rutherford and Mourez, 2006; van den Berg, 2010). More importantly, these autotransporters are shown to be able to translocate single-chain antibody molecules onto the bacterial surface (Pyo et al., 2009; Veiga et al., 1999, 2003).

In this report, we have used the gram-negative bacteria for surface display of anti-HIV-1 antibody molecules. The autotransporter, an immunoglobulin A (IgA) protease gene (IgAP) of *Neisseria gonorrhoeae,* was used because its C-terminal domain has been shown to efficiently translocate the passenger domain at its N-terminal (Dautin and Bernstein, 2007; Pohlner et al., 1987). We therefore employed this gram-negative bacterial autotransporter for surface display of a potent broadly neutralizing antibody (VRC01) against HIV-1 infection. VRC01 is a potent neutralizing antibody isolated from an HIV-1 patient, which can neutralize about 90% of HIV-1 isolates tested (Wu et al., 2010). VRC01 binds to the CD4-binding site (CD4-BS) and can mimic CD4-binding to gp120 (Zhou et al., 2010). We have generated the single-chain variable fragment (scFv) of VRC01 antibody (scFv-VRC01) and displayed it on the surface of *E. coli* to test its ability to inhibit HIV-1 infection.

Results

Design of the scFv-VRC01 surface-display constructs

The scFv-VRC01 was designed using a two-step approach. The first was the designing of the single-chain (scFv) VRC01 antibody domain for expression. The VRC01 antibody gene was used to generate the single-chain antibody (scFv). A linker (-GGGGSG-GGGSGGGGS-) was used to link the heavy chain (VH) and light chain (VL) gene fragments. Two E-tags were inserted into the recombinant gene; one was located between the β-barrel domain and the single-chain antibody, another was added to the N-terminus of the single-chain antibody (Fig. 1A). The resulting recombinant protein would display the His-tag at the C-terminus when expressed in the pET22b vector, and will be 257 amino acids in length with an expected molecular weight of about 27 kDa. The designed peptide was codon-optimized and synthesized for the *E. coli* expression system. The second step was to link scFv-VRC01 fragment to the translocator β-barrel domain (C-IgAP) from bacterial (*N. gonorrhoeae*) autotransporter (434aa), which will then generate a fusion protein (scFv-VRC01-β-barrel domain (C-IgAP)) of about 75 kDa. The proposed structural model of the fusion recombinant protein molecule is shown in Fig. 1B. The scFv-VRC01 fusion upon expression is then expected to be displayed on the surface of the bacterial cell and bind to gp120 on the surface of the HIV-1 virion to inhibit viral infection.

Expression of scFv-VRC01 antibody in E. coli

The synthesized single-chain VRC01 antibody (scFv-VRC01) gene was first cloned into the pET28b expression vector to test for

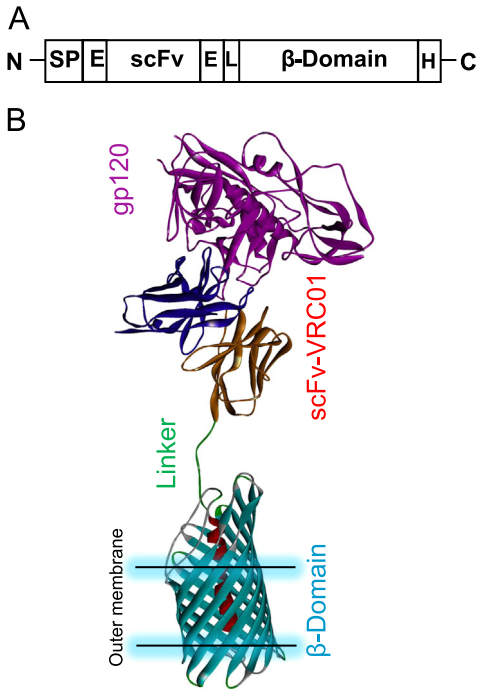

Fig. 1. Construction of fusion protein of single-chain antibody VRC01 and autotransporter β-domain from *N. gornohoeae*. (A) Schematic representation of the fusion protein construct for surface display. Sp, signal peptide; E, E-tag; L, linker (GSG); scFv, single-chain variable fragment; β-domain, the c-terminal part of autotransporter serine proteinase gene of *N. gornohoeae*. H, His-tag. (B) Three dimensional (3D) model of the fusion protein for surface display and its binding to gp120. The linker is GSG plus the E-tag, β-barrel domain (PDB 1UYN (van den Berg, 2010)), scFV-VRC01 and gp120 of HIV-1 (PDB 3NGB).

expression of the scFv-VRC01 protein with the predicted molecular weight of about 27 kDa. As shown in Fig. 2A, a band of 27 kDa could be detected in total bacterial lysates upon induction by IPTG, but was absent in both un-induced lysates or in the supernatant of induced samples. This suggests that the single-chain VRC01 antibody protein molecule could be expressed in *E. coli*, and found mostly in the insoluble fractions of the bacterial lysates. The recombinant protein was then further purified using the Ni-NTA column and the presence of the protein was confirmed by Western blot analysis using anti-His-tag antibody. An example of the blot is shown in Fig. 2B, confirming that most of the expressed protein could be found in the insoluble fraction of the bacterial lysate.

We then further characterized the expressed single-chain antibody VRC01 by determining whether it could be refolded into a native conformation, and Far-Western blotting was used to analyze whether it could bind gp120. This was carried out using anti-gp120 antibody to detect gp120 that was captured by the refolded scFv-VRC01, and the result is shown in Fig. 2D. The gp120 molecule was found to be bound by the refolded scFv-VRC01 on the membrane, resulting in the presence of a 27 kDa band which corresponds with expected size of the scFv-VRC01 antibody molecule and with the size of purified scFV-VRC01 shown by Western blot analysis using anti-His-tag antibody (Fig. 2C). Our results thus confirmed that the detected 27 kDa recombinant protein was indeed scFv-VRC01 and when refolded, could bind gp120.

Inhibition of HIV-1 by the scFv-VRC01 antibody

Since the recombinant scFv-VRC01 can bind gp120 as determined by Far-Western blotting analysis, this suggested that the refolded SCFV-VRC01 can possibly bind and neutralize HIV-1. We then tested whether the expressed single-chain antibody VRC01 had neutralizing activity for different HIV-1 subtype strains. We tested