Non-neutralizing antibody therapeutics to eliminate HIV-infected cells *in vitro* and *in vivo via* ADCC

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BACKGROUND

In HIV-1 infection, a significant fraction of antibodies (Abs) is induced to epitopes that are obscured on the unliganded Env trimer, either at the surface of infected cells or on viral particles. A prime example of this are Abs targeting CD4 induced (CD4i) epitopes, exposed only upon CD4 interaction. Most CD4i specific Abs are non-neutralizing or have weak neutralization activity. Their FcR activities, on the other hand, are usually very potent but dependent on CD4i epitope exposure. Interestingly, some CD4i epitopes, including co-receptor binding site (CoRBS), or epitopes mapping to gp120 inner domain constant region 1 and 2 (Cluster A), are the most conserved Env regions (**Figure 2**). Consequently, their potential utility as targets for Ab-based approaches is high, provided the challenge of occluded exposure is overcome.

METHODS

We utilized structure-based engineering to develop CoRBS or Cluster A Abs into Ab-CD4 hybrids or Ab-CD4 mimetic (CD4 mc) conjugates. In an Ab-CD4 hybrid, Ab is linked via a flexible $(G_4S)_6(G_4T)_2$-linker (~152 Å long) to CD4 (the d1d2 domain of CD4, residues 26-208 (1), whereas in Ab-CD4mc conjugate the CD4 is replaced by small compound CD4 antagonist, CJF-III-288 and linked to the Ab by a $(PEG)_{23}$-linker. Ab-CJF-III-288 conjugate is produced using a tRNA suppressor system where an unnatural aa, Azido-L-phenylalanine (AzF), is first introduced at a specific site of the Ab (**Figure 1**) and linked to DBCO-$(PEG)_{23}$-CD4mc through copper–Free click Chemistry (SPAAC) ligation.

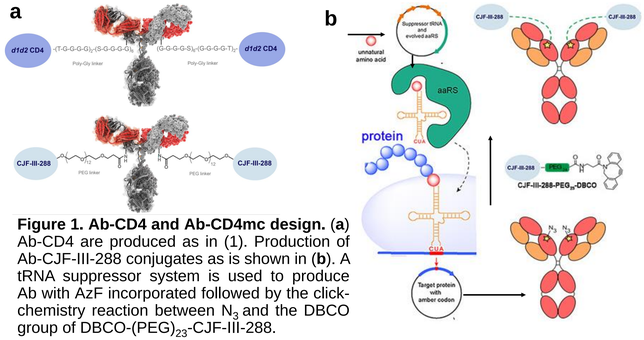


Figure 1. Ab-CD4 and Ab-CD4mc design. (**a**) Ab-CD4 are produced as in (1). Production of Ab-CJF-III-288 conjugates as is shown in (**b**). A tRNA suppressor system is used to produce Ab with AzF incorporated followed by the click-chemistry reaction between N_3 and the DBCO group of DBCO-$(PEG)_{23}$-CJF-III-288.

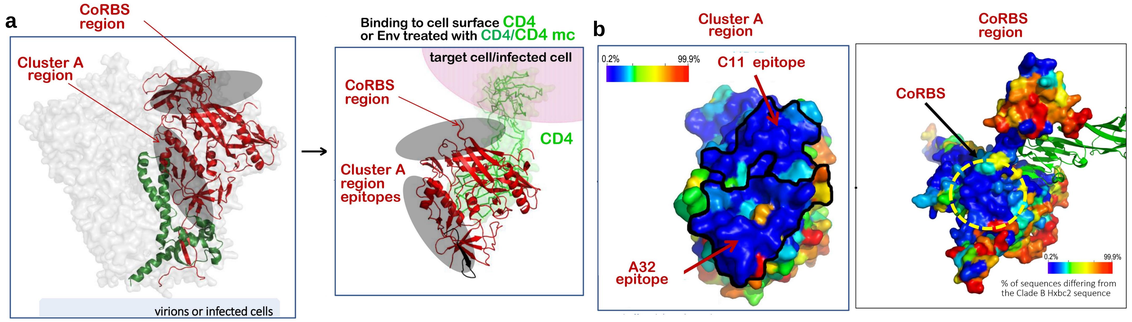


Figure 2. Cluster A and CoRBS epitope exposure and conservation. (**a**) Co-localization of Cluster A and CoRBS epitopes within the unliganded HIV-1 trimer. Structural rearrangements (post-CD4 binding) are required for Cluster A/CoRBS epitope formation/exposure. (**b**) Sequence conservation. The molecular surface is displayed over gp120 and colored in gradient based on a sequence conservation (residues differing by 0.2-7% and 87-99.9% from the Hxbc2 sequence colored dark blue to red, respectively).

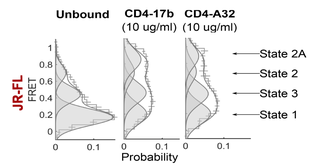


Figure 3. Ab-CD4 are capable of triggering HIV-1 Env to the 2A Env state Histograms of FRET values resulting from HMM analysis observed for HIV-1 JR-FL Env in the presence and absence of bound Ab-CD4s.

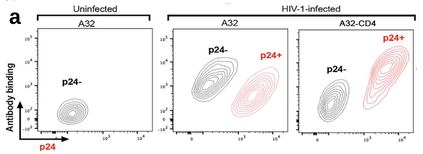


Figure 4. Ab-CD4 hybrids effectively recognize and eliminate HIV-1-infected cells through ADCC. (**a**) Primary CD4+ T cells infected with JRFL were stained with A32 or A32-CD4. Infected cells were identified by intracellular p24 staining. (**b**) Ab-CD4 mediated ADCC of CD4+ T cells infected with Tier 2 JRFL. Statistical significance was tested using an unpaired t-test (**, P < 0.01, ***, P < 0.001).

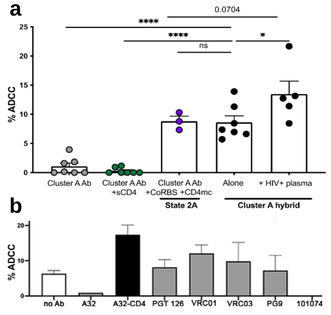


Figure 5. Ab-CD4 are capable of potent ADCC alone and synergize with Abs present in plasma of HIV-1 infected individuals ADCC of HIV-1 JRFL-infected primary CD4+ T cells in the presence of HIV+ plasma by (**a**) Ab-CD4 hybrid compared to the State 2A cocktail and (**b**) bnAbs.

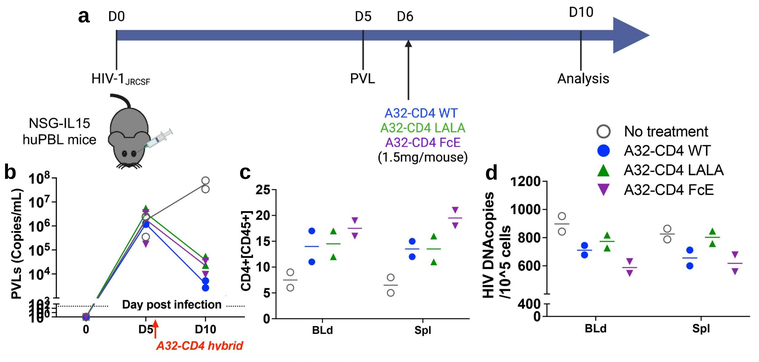


Figure 6. *In vivo* activity of A32-CD4 hybrids in hu-mice. (**a**) Experimental outine. HIV-1JRCSF-infected NSG-IL15 hu-PBL mice were administrated indicated A32-CD4 variants at day 6 post-infection. Mice were bled at day 0, 5 and 10 post-infection for plasma viral load (PVL). At day 10 post-infection, tissues were harvested, andcells isolated for real-time PCR analysis of HIV DNA levels. (**b**) PVL was measured by quantitative real-time PCR (Limit of detection =300 copies/mL, dashed line). (**c**) % CD4+ T cells was measured by flow cytometry in indicated tissues. (**d**) CD4+ T cells were purified from peripheral blood and spleen for real-time quantitative PCR analysis of HIV DNA level.

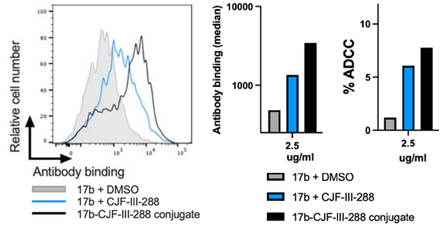


Figure 7. 17b-CJF-III-288 conjugate effectively recognizes and eliminates HIV-1-infected cells through ADCC (a) Primary CD4+ T cells infected with CH058TF were stained with 17b-CJF-III-288. Infected cells were identified by intracellular p24 staining. **(b)** 17b-CJF-III-288 binding and ADCC of CD4+ T cells infected with CH058TF.

RESULTS

17b-CD4 and A32-CD4 directly stabilize the State 2A Env conformation (**Figure 3**) and are capable HIV-1 infected cells recognition and elimination through ADCC mechanism (**Figure 4**). Interestingly, A32-CD4 hybrid is also able to synergize with Abs present in HIV+ plasma in mediating ADCC of infected cells. In this format A32-CD4 hybrid outcompetes the best in class broadly neutralizing Abs (bnAbs) (**Figure 5**). Results in humanized mice (hu-mice) show that A32-CD4 can control HIV-1 replication *in vivo,* as well as to reduce the level of integrated HIV DNA in the blood and in the spleen in an FcR-dependent manner (**Figure 6**). Finally, we show that the CD4 moiety in Ab-CD4 hybrid can be replaced by the small molecule CD4mc. Our data confirm *in vitro* the capacity of an Ab-CJF-III-288 conjugate utilizing Abs of a CoRBS specificity *in vivo* testing is ongoing.

CONCLUSIONS

Our data provide a solid proof of concept that bispecific molecules consisting of ‘an opener’ attached through an appropriate linker to a CD4i Ab are capable of cooperative action to stabilize the State 2A conformation and mediate Fc-effector function *in vitro* and *in vivo.* Furthermore, in addition to acting alone, our prototype Ab-CD4s are also capable of unleashing the ADCC potential of Abs present in HIV-1+ plasma. Altogether, these data confirm the utility CD4i Abs-CD4/CD4mc as therapeutics capable of overcoming a major obstacle in eliminating HIV-1 infected cells via ADCC that could be utilized in strategies to reduce or eliminate the viral reservoir in people living with HIV.

Reference: (1) Richard, Nguyuen at al., mBio. 2021 Oct 26;12(5):e0140521. doi: 10.1128/mBio.01405-21. Epub 2021 Sep 28. PMID: 34579568

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