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Enhancing ADCC Responses in HIV-1 Vaccines: CD4 Incorporation Into Virus-Like Particles (VLPs)

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BACKGROUND

In RV144, antibody-dependent cellular cytotoxicity (ADCC) correlated with reduced HIV-1 infection risk, underscoring the importance of Fc-mediated immunity. We describe a VLP-based HIV-1 vaccine prototype incorporating CD4 to expose normally hidden CD4-inducible (CD4i) epitopes, increasing binding of CD4i antibodies 2–5-fold while largely preserving broad neutralizing antibody (bNAb) binding. Notably, FcγRIIIa activation increased 2.7-fold, potentially enhancing Fc-mediated immune responses.

METHODS

VLPs were produced by transient co-transfection in 293F cells (**a**); size by Nanoparticle Tracking Analysis (**b**); Env incorporation by Western blotting (**c**) and immunoelectron microscopy (**d**); antigenicity by VLP ELISA (**e**) and FcγRIIIa activation as a proxy for ADCC (**f**).

A CD4 VLP vaccine prototype promotes CD4i epitope exposure and FcγRIIIa activation to boost Fc-mediated immunity.

RESULTS

CD4 incorporated efficiently into VLPs without modifying particle size and moderately reducing Env incorporation (18–43%). CD4/VLPs increased CD4i antibody binding by 2 to 5-fold while maintained bNAbs binding with moderate affinity loss (1.3–4.2-fold). In addition, CD4/VLPs showed an increase in FcγRIIIa activation 2.7-fold per spike.

Impact of CD4 incorporation on HIV-1 Env VLP structure, antigenicity, and effector function. (**a-b**) HIV-1 Env VLPs (AC10.29, ConM, and 763) produced in 293F cells in the absence (a) or presence (b) of CD4 were analyzed for particle size and distribution by nanoparticle tracking analysis (NTA). (**c**) Env and CD4 incorporation into VLPs was assessed by SDS–PAGE and Western blotting; gp160, gp120, CD4, and p55 were detected using 447-52D (gp120), anti-p24 (p55), and anti-CD4 antibodies. (**d**) Surface coexpression of HIV-1 Env and CD4 visualized by immunoelectron microscopy using anti-gp120 antibodies and anti-hCD4 antibodies [25 and10-nm (red triangles) gold labeled secondary antibodies, respectively]. (**e**) Binding of gp120-normalized CD4− (open circles) and CD4+ (filled squares) VLPs to CD4i antibodies (17b, 48d, A32) and to bNAbs (2F5, VRC01, PG16) measured by ELISA. (**f**) Antibody binding expressed as area under the curve (AUC) ratios of CD4+ versus CD4− VLPs (mean ± SD, n = 3). (**g**) FcγRIIIa activation in Jurkat-Lucia™ NFAT-CD16 cells, as a proxy for ADCC, using pooled HIV-positive and HIV-negative sera; data are expressed as RLUs normalized to gp120 content.

CONCLUSIONS

The development of an effective HIV-1 vaccine remains a major global health challenge, and growing evidence indicates that Fc-mediated effector functions, particularly ADCC, contribute to protective immunity even in the absence of potent neutralizing responses.

Here, we describe a VLP-based vaccine platform that enhances CD4i epitope exposure through incorporation of membrane-anchored CD4. This approach increased binding of CD4i-targeting antibodies and enhanced FcγRIIIa engagement, supporting the induction of ADCC-associated effector functions previously linked to reduced HIV-1 infection risk. Importantly, CD4 incorporation caused only moderate, Env-dependent reductions in bNAb recognition. Although antibody binding varied by Env variant and epitope specificity, key neutralizing determinants were largely preserved, indicating that CD4i epitope exposure can be achieved while maintaining neutralization-sensitive sites. These results suggest that CD4 incorporation can be tuned to balance enhanced Fc-mediated activity with retention of bNAb recognition.This membrane-tethered CD4 VLP strategy complements existing approaches to enhance Fc-mediated immune responses and may offer advantages over soluble CD4 or CD4-mimetic designs by promoting more physiologically relevant Env–CD4 interactions and multivalent epitope display.

While *in vivo* immunogenicity and protective efficacy remain to be determined, and further optimization of Env and CD4 stoichiometry may be required, this platform introduces a flexible immunogen design element with potential to inform next-generation HIV-1 vaccine strategies focused on Fc-mediated immunity.

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Our research introduces a vaccine design that exposes hidden parts of HIV, allowing antibodies to more effectively trigger immune cells that eliminate infected cells. At the same time, it preserves key targets of protective antibodies, supporting the development of more balanced and effective HIV vaccines.