Enhancing HIV Vaccine Efficacy via Langerhans Cell-Targeted Env trimers

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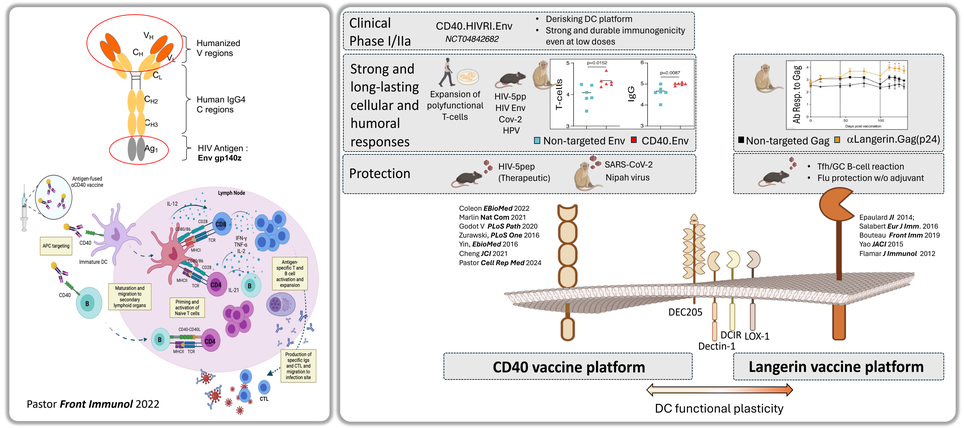
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BACKGROUND

The main goal for the development of an HIV-1 vaccine remains the induction of protective antibodies. New modes of antigen delivery are warranted to improve the quality of immune responses.

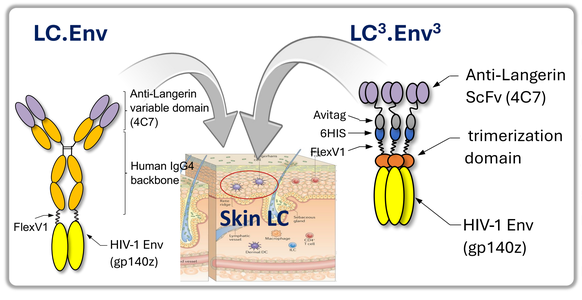
A rationale approach is to **target antigen to specific receptors expressed on the surface of antigen-presenting cells (APC)** via fused monoclonal antibodies (mAb) to promote cellular and humoral responses.



HYPOTHESIS

Previous studies have shown success in targeting skin Langerhans cells (LC) with anti-Langerin mAbs fused to HIV-1 Envelope **(LC.Env), inducing antigen-specific humoral responses in mice and human LC:T/B co-cultures** (Kervevan ***PLoS Path*** 2021)

LC are valuable targets for inducing HIV Env-specific Ab

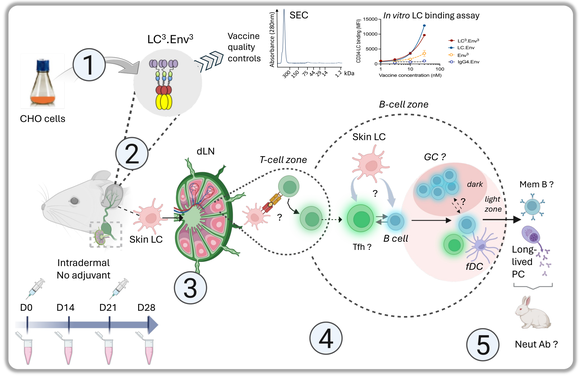


Here, we aimed to refine LC targeting by designing three Env monochains ($LC^3.Env^3$) instead of two (LC.Env) mimicking natural Env conformation.

We aim to investigate **whether these new trimeric Env constructs could stimulate germinal center (GC) and Tfh cell reactions**, ultimately leading to the production of **Env-binding IgG and HIV neutralizing antibodies (NeutAb)** ***in vivo***

METHODS

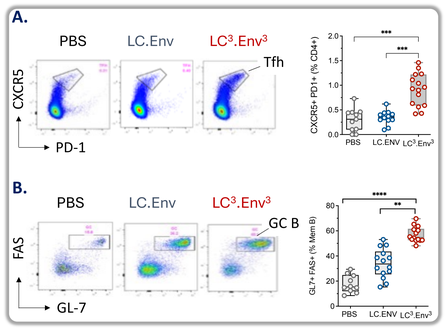
1. LC.Env and $LC^3.Env^3$ were produced in CHO cells, and controlled for quality
2. mice were **intradermally** immunized with $LC^3.Env^3$ or control LC.Env mAb (5 mcg of Env antigen) at day (D) 0 and D21, without adjuvant.
3. **Antibody (magnitude and affinity)** and cellular responses were assessed post-prime (PP) and post-boost (PB) using Luminex and FACS.
4. **GC/Tfh reactions in draining lymph nodes (dLN) were monitored through immunofluorescence** at various times.
5. Additionally, rabbits were subcutaneously immunized with 50 mcg $LC^3.Env^3$ without any adjuvant, **and serum NeutAb levels** were evaluated.



Targeting HIV-1 Env trimers to Langerhans cells ($LC^3.Env^3$) enhances HIV-1 Env antibody responses, rapidly inducing potent, structured germinal centers.
New delivery of antigen designed for the induction of NeutAbs may improve the induction of protective antibodies.

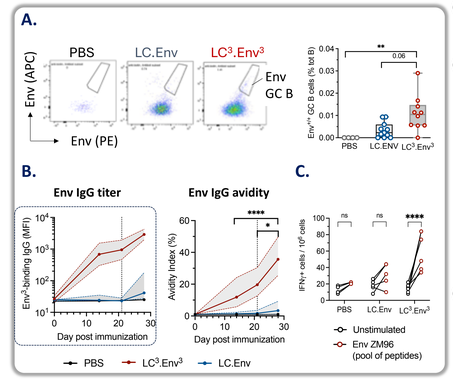
RESULTS

Fig-1. $LC^3.Env^3$ elicits robust Tfh/GC responses in dLN



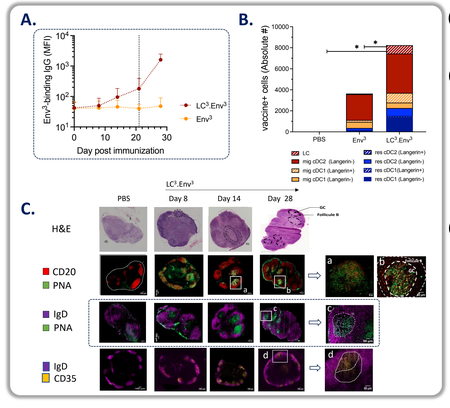
(A) ID immunization in mice with $LC^3.Env^3$ or LC.Env, following a prime-boost schedule, led to notable **Tfh cell** (PD-1+ CXCR5+ CD4+ T cells) and
(B) induction and expansion of **GC B cells** (GL-7+/FAS+ B cells) post-boost, as observed in representative dot plots and percentages.

Fig-2. $LC^3.Env^3$ improves LC targeting



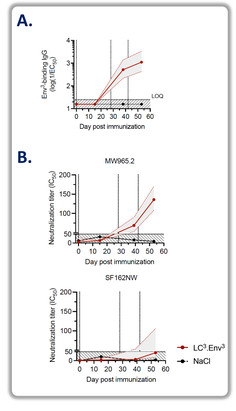
(A) Env-specific GC B cells were identified with statistical significance.
(B) Luminex analysis showed **elevated Env-specific IgG** levels post-vaccination, peaking at day 28. Avidity index calculations demonstrated **sustained responses** induced by $LC^3.Env^3$ over subsequent time points.
(C) $LC^3.Env^3$ vaccinated mice exhibited **INF-γ T cell responses** in dLN, quantified via ELISpot.

Fig-3. $LC^3.Env^3$ enhances APC-uptake and induces GC formation



(A) $LC^3.Env^3$ induced higher Env IgG titers compared to non-targeting Env^3 HIV-1 trimer, with geometric means (±SD) presented.
(B) Fluorescent $LC^3.Env^3$ and Env^3 were intradermally administered in mice, and draining lymph nodes were analyzed. **$LC^3.Env^3$ exhibited higher uptake by migratory dendritic cells expressing Langerin receptor**, confirmed by FACS analysis. Statistical significance was determined using the 2-way ANOVA test.
(C) $LC^3.Env^3$ vaccination resulted in distinct histological changes in draining lymph nodes, observed through **H&E staining and immunofluorescence.** The images highlighted the **development of germinal center (GC) structures**, confirmed by CD20, IgD, and PNA markers. Noteworthily, at Day 28, fDC (CD35+) polarized arrangement that defines the germinal center's light zone.

Fig-4. $LC^3.Env^3$ induces tier-1 NeutAbs



(A) New Zealand white rabbits, subcutaneously immunized with $LC^3.Env^3$, showed elevated **Env-specific IgG,** measured by ELISA.
(B) Neutralizing assays indicated the sera's capacity to **neutralize tier-1 HIV-1 viruses, presented as Log IC_{50},** with a dashed zone indicating low or non-neutralizing activity below a titer of 50.

CONCLUSION

This study underscores that HIV Env antigen can be **adaptively targeted to LC as a trimer, intensifying both the magnitude and quality of humoral responses without using any adjuvant** and after only two shots.
In addition, the **strategy of targeting SOSIP-like trimeric Env to LC holds substantial promise** for eliciting broadly neutralizing antibodies (bNAb) against HIV and will require an optimization of the vaccination regimen.

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