



Laboratory of
Experimental Virology

Enhancing the immunogenicity of HIV-1 envelope glycoprotein trimers by fusion to CD40 ligand

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How does the immunogenicity of gp120 compare to other antigens?

- Relatively high amounts of antigen and multiple immunizations needed to induce any Ab response (e.g. 300-600 μg gp120 vs 20 μg HBsAg)
- Half life of the Ab response in gp120 vaccinees is short ($T_{1/2}$ = 30-60 days)
- Difficult to elicit sustained T-helper and memory B cell responses
- Response to gp120 is unusually Th2-biased
- Mannoses on gp120 induce immunosuppressive IL-10 response (Shan *et al.* PLoS Pathog. 2007)



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**Can we improve the immunogenicity
of Env by fusion to CD40 ligand?**

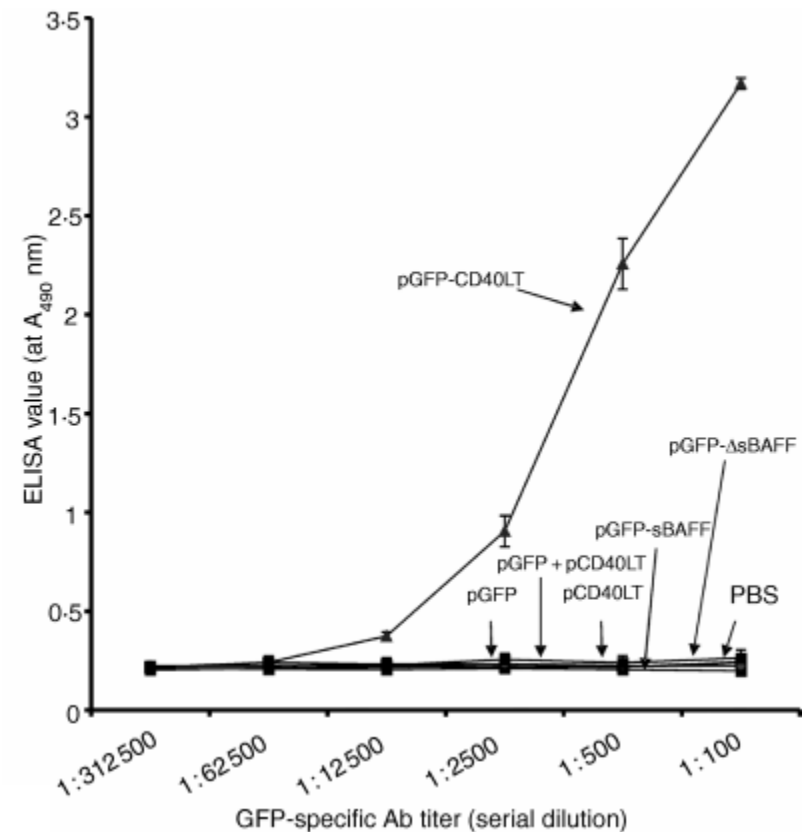


CD40 Ligand (CD154)

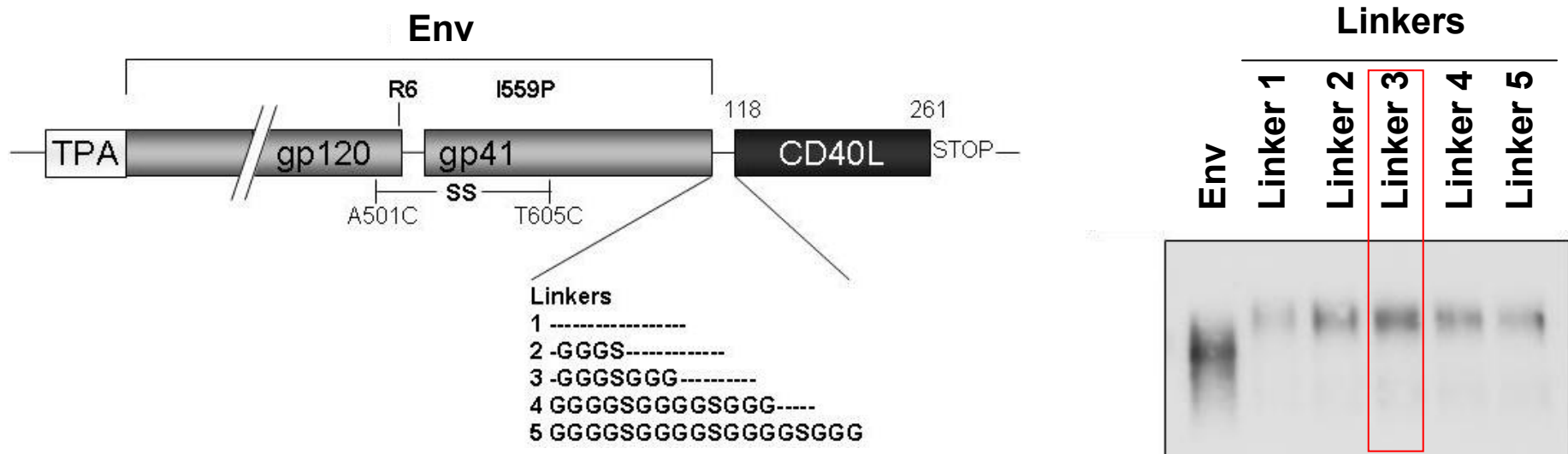
- Natural ligand for CD40
- Predominantly expressed on activated CD4+ T-helper lymphocytes
- Binds to CD40 on Dendritic cells and B cells (part of T help)
- Promotes:
 - B-cell growth
 - Differentiation to antibody-secreting plasma cells and memory B cells
 - Selection in germinal centers
 - Isotype switching
 - Antigen presentation
 - cytokine production (e.g. IL-6)
- Safe for use in humans (Vonderheide *et al.* J. Clin. Onc. 2001)

Fusion of CD40L to GFP improves GFP-specific antibody titers

- Synergistic Ab induction by antigen-CD40L fusion protein
→
(Li *et al.*, Immunology 2005)
- Improved immunogenicity of a **self (tumor) antigen** by fusion to CD40L
(Huang *et al.*, Int J cancer 2004)



Design of Env-CD40L fusion protein (1)

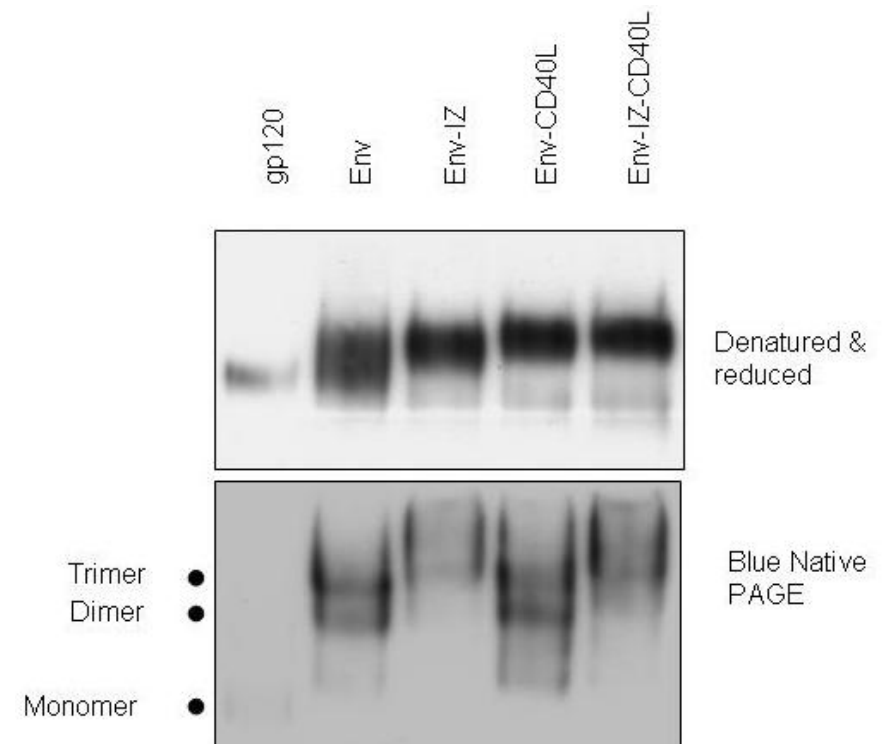
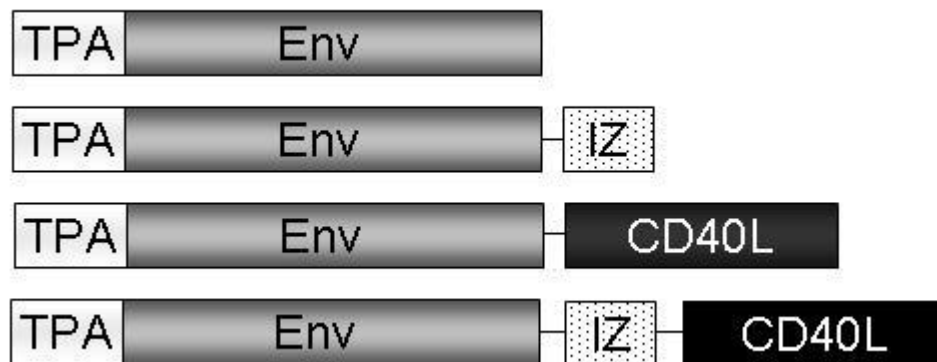


- Linker 3 provides optimal spacing between Env and CD40L

Design of Env-CD40L fusion protein (2)

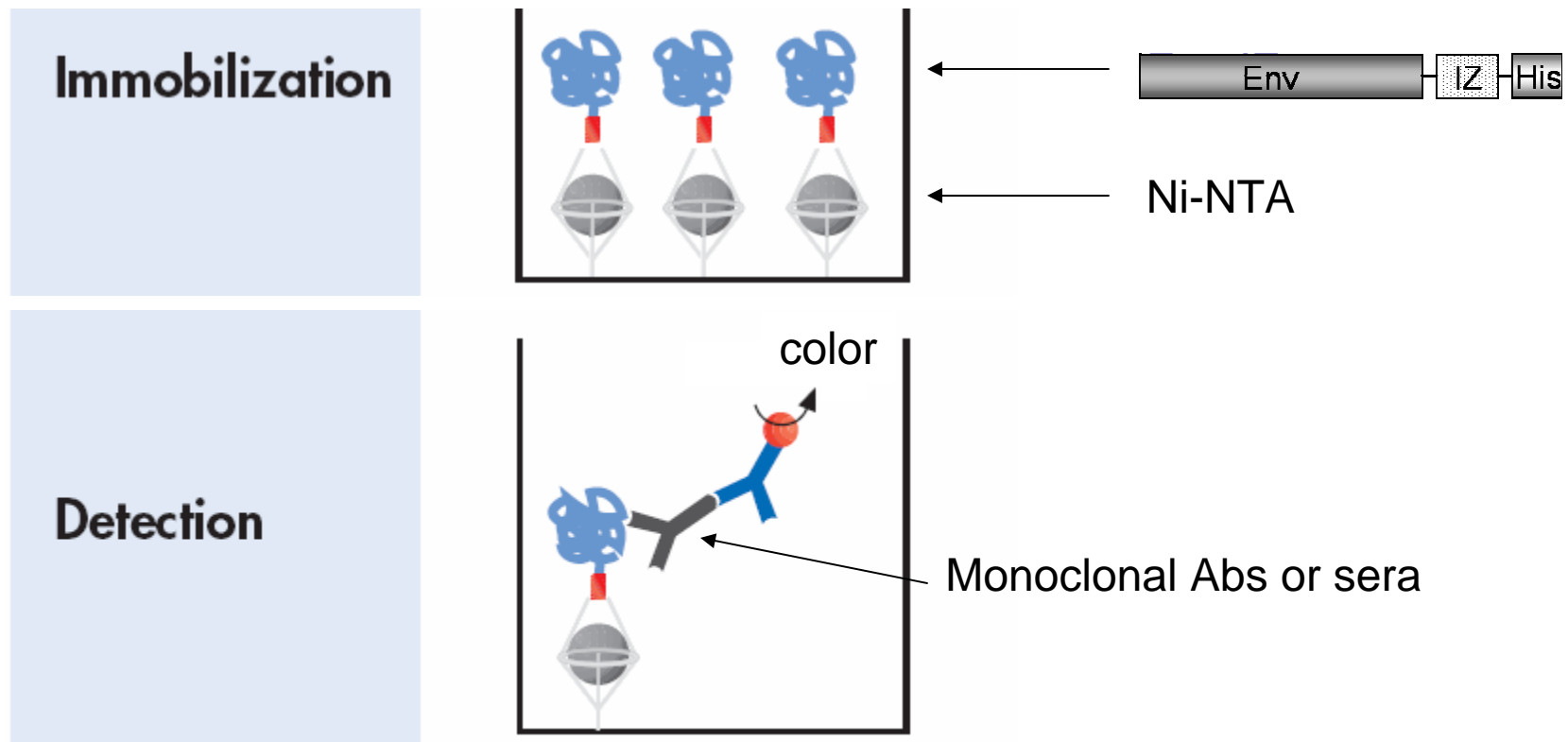
- Both Env and CD40L need to trimerize
- Both soluble Env trimers and soluble CD40L trimers are unstable

→ Add trimerization motif
– Isoleucine zipper (IZ)



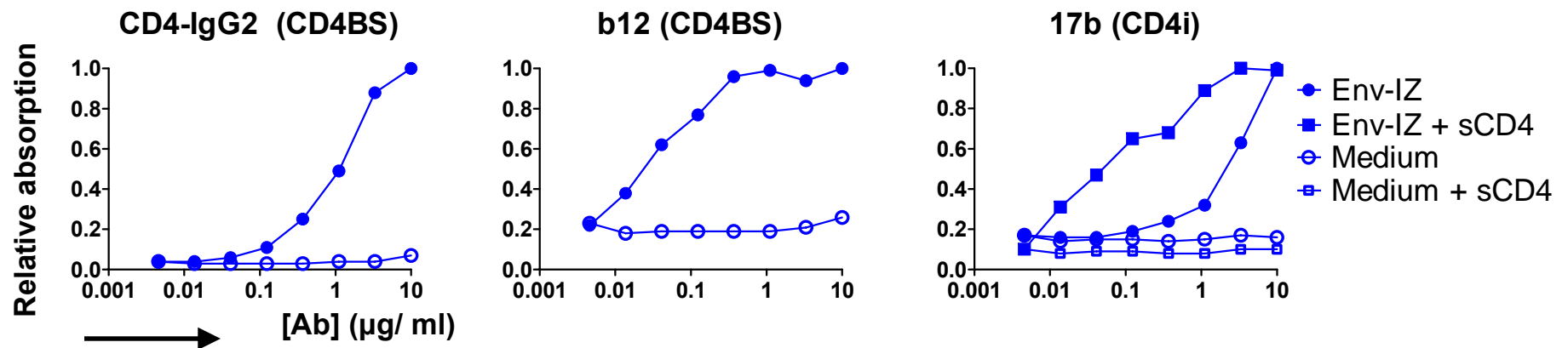
Trimer probing by ELISA

- Addition of C-terminal His-Tag
- Ni²⁺-coated ELISA plates
- Probe with monoclonal Abs and receptors



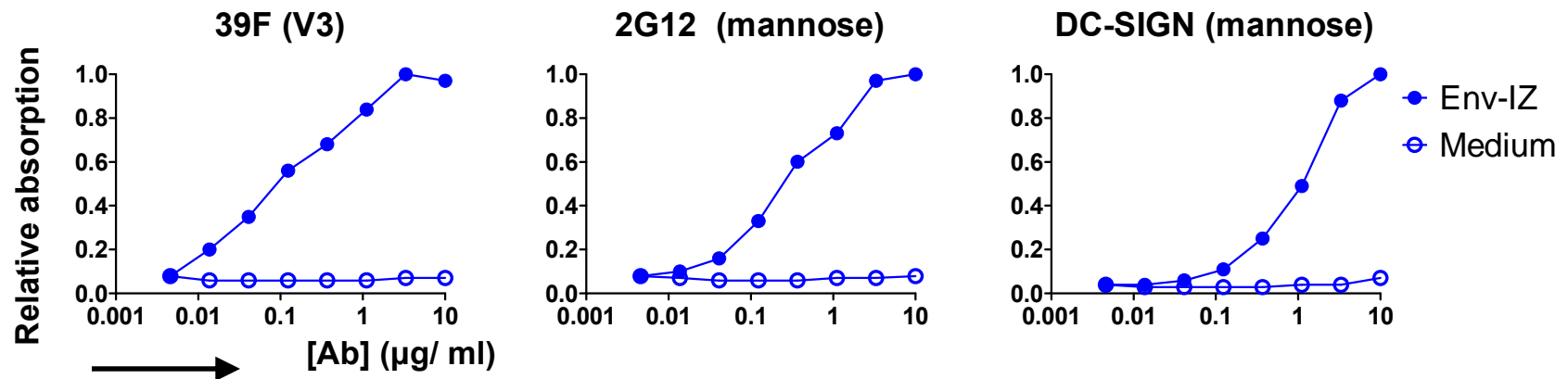
Trimer probing (1): CD4BS

- CD4BS is exposed and functional
- Env-IZ is able to undergo CD4-induced conformational changes



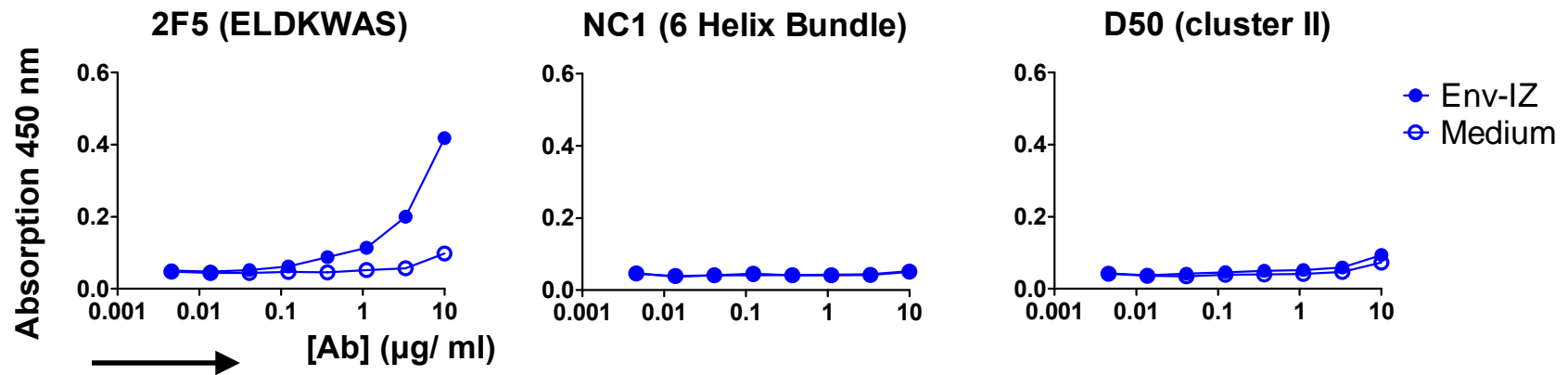
Trimer probing (2): V3 and glycans

- Neutralizing epitopes are recognized
- Mannoses on Env-IZ are recognized by DC-SIGN and 2G12



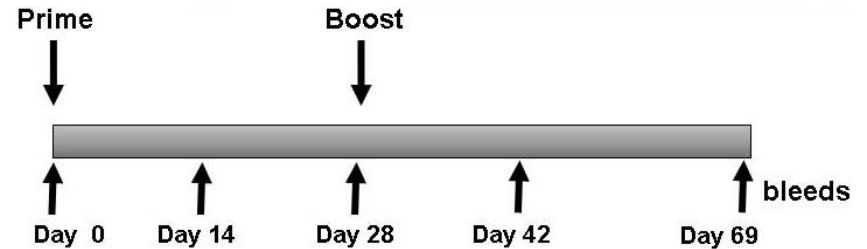
Trimer probing (3): gp41

- Neutralizing epitopes are recognized
- Non-neutralizing epitopes are not recognized



Immunogenicity of Env-IZ-CD40L (1)

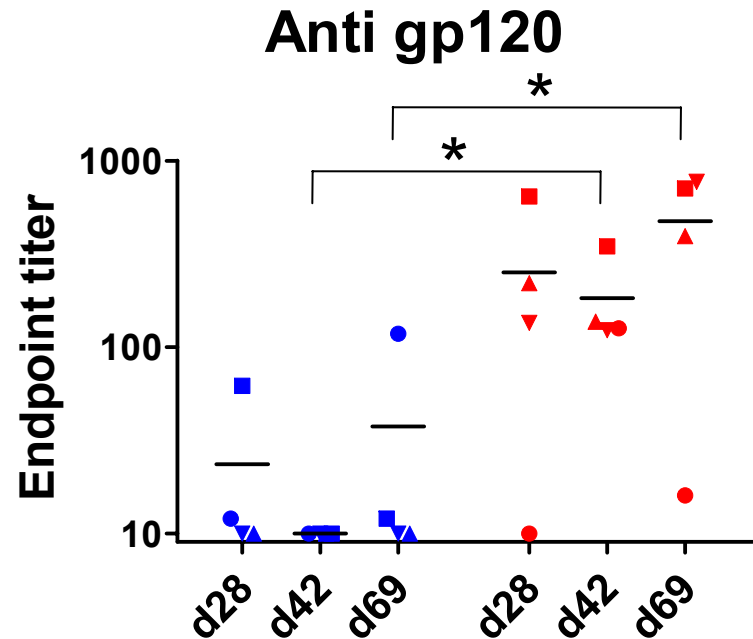
- Balb/C mice
- 2 groups of 4 mice:
 - Env-IZ
 - Env-IZ-CD40L



- DNA immunizations

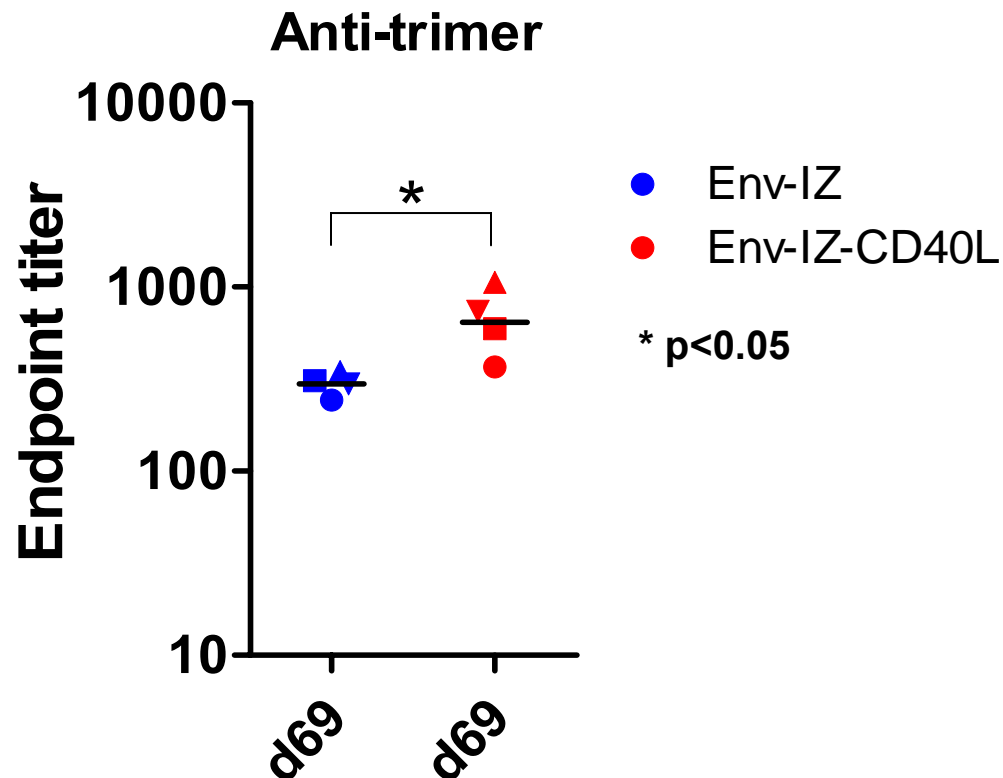
- Env-IZ
- Env-IZ-CD40L

* $p < 0.05$



Immunogenicity of Env-IZ-CD40L (2)

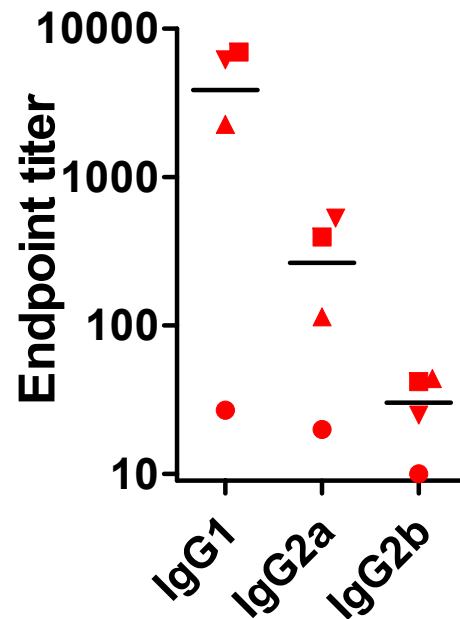
- Induction of trimer-binding Abs



Immunogenicity of Env-IZ-CD40L (3)

- Induction of various Env-specific IgG subclasses → balanced Th1/Th2 response

Anti-gp120 subclasses



Conclusions

- We were able to generate a trimeric Env-CD40L fusion protein
- Ni²⁺-ELISA is suitable for probing trimer surface
- Env-IZ is recognized by conformational Abs, CD4 and DC-SIGN
- Env-IZ can undergo CD4i conformational changes
- Fusion to CD40L enhances Env immunogenicity
- Induction of various Env-specific IgG subclasses
→ balanced Th1/Th2 response
- Next: neutralization experiments with rabbit sera

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