A TRIMERIC HIV-1 GP140-BAFF FUSION CONSTRUCT ENHANCES MUCOSAL ANTI-TRIMERIC HIV-1 GP140 IGA IN MICE

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Background

• A safe and effective HIV-1 vaccine is needed to ultimately control HIV-1 pandemic

• Broadly neutralizing antibodies (bNAbs) can prevent HIV-1 infection
  (sterilizing immunity) and thus are the holy grail for HIV-1 vaccine development

• Non-broadly neutralizing antibodies (nbNAbs) can also prevent HIV-1 infection

• To elicit protective antibodies (bNAbs and nbNAbs), the target antigen, HIV-1 Env, should mimic the native trimer conformation.
Klein JS et al. Plos Patho, 2010, 6:e1000908
https://www.aidsreagent.org/program_info.cfm
Background

• HIV-1 predominantly transmits through genital/rectal mucosa.

• Mucosal IgA is the dominant Ig subtype at most mucosal surface (except genital mucosa) and is vital for prevention of microbial transmucosal infections, including HIV-1.

• HIV-1 Env is weak in immunogenicity and needs potent adjuvants to elicit strong and long-lasting Ab responses.

• Three TNFSF members, CD40L, BAFF (B cell activating factor of the TNF family), APRIL (a proliferation-inducing ligand), are costimulatory molecules for antibody responses through promoting B cell proliferation and survival, Ig isotype switch (IgM→IgG and IgA), and somatic hypermutation (affinity maturation).
Hypothesis

A trimeric fusion construct of HIV-1 Env and APRIL/BAFF/CD40L (Env-A/B/C trimer) can improve anti-HIV-1 Env antibody responses.
Trimerized fusion protein constructs

control constructs

- flexible GGGSGGGG linker
- FLAG: 3xFLAG tag
- TD: trimerization domain
- THD: TNF homology domain
Questions

• Will the fusion constructs form trimer?
• Will the fusion constructs keep the native conformation of HIV-1 Env?
• Can the fusion constructs enhance antibody responses against HIV-1 Env, esp. at mucosal surface?
Transfect HEK293T cells

Supernatant subjected to SDS-PAGE, Blue native PAGE, immunoprecipitation followed by Western blot
Fusion constructs form trimers and keep HIV-1 Env native conformation

Blue native PAGE

Trimer

Dimer

Monomer

1236kDa

1048kDa

720kDa

480kDa

242kDa

146kDa

Controls

Env Antibodies

Staining materials

Negative

gp140-FLAG

gp140

gp140-APRIL

gp140-BAFF

gp140-CD40L

gp140-FOLDON

gp140

Credit: Clayton K, et al.
Questions

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• Will the fusion constructs keep the native conformation of HIV-1 Env?
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Vaccination regimen

- 6 groups (4 mice/group):
  - gp140
  - gp140-FOLDON
  - gp140-CD40L
  - gp140-BAFF
  - gp140-APRIL
  - Naïve (PBS)
- DNA: 100 µg in 100µl PBS/mouse/vaccination, 50µg(µl) per hing leg muscle per mouse
- Protein: 20µg in 100 µl PBS mouse/vaccination, i.p.

Sampling: Fecal pellet, vaginal lavage, blood sera

Sacrifice mouse Sampling: Fecal pellet, vaginal lavage, blood sera, spleen

Detection of Abs: Kinetic ELISA using gp140-FOLDON as coating antigen
Kinetic ELISA

- Take OD\(_{650}\) reading every 15s for the first 3 min after adding substrate, and calculate slope (\(\Delta mOD_{650}/\text{min}\)) from linear regression.
- More accurate in Ab quantification than endpoint ELISA: slope is proportional to concentration of antigen/antibody during initial stage of reaction.
- No need to do serial dilutions of samples.
- Standardization of mucosal gp140-specific Ab: \(\Delta mOD_{650}/\text{min/total IgG or IgA} (\Delta mOD_{650}/\text{min/ng·mL}^{-1}).\)
HIV-1 trimeric gp140-specific antibody responses - sera

Sera gp140-specific IgG

Sera gp140-specific IgA

- Naive (PBS)
- gp140-APRIL
- gp140-BAFF
- gp140-CD40L
- gp140-foldon
- gp140

Week 9, Week 11, Week 13
HIV-1 trimeric gp140-specific antibody responses - vaginal lavage

**Vaginal gp140-specific IgG**

**Vaginal gp140-specific IgA**

- **Week 9**
- **Week 11**
- **Week 13**

**Graphs:**
- Vaginal gp140-specific IgG
- Vaginal gp140-specific IgA
- Data points for each week and treatment group.
HIV-1 trimeric gp140-specific antibody responses-fecal pellet

**Fecal gp140-specific IgG**

**Fecal gp140-specific IgA**

- **Naive (PBS)**
- **gp140-APRIL**
- **gp140-BAFF**
- **gp140-CD40L**
- **gp140-foldon**
- **gp140**
Conclusions

• Fusion constructs, gp140-ARPII/BAFF/CD40L, form trimers and keep native conformation of HIV-1 Env.

• gp140-BAFF can enhance trimeric HIV-1 Env-specific antibody responses, esp. mucosal IgA responses.

• gp140-APRIL and gp140-CD40L can not enhance trimeric HIV-1 Env-specific antibody responses.
Ongoing experiments and future directions

- HIV-1 neutralization
- Other vaccination platforms using gp140-BAFF as immunogen? (microneedle, nanoparticles, etc.)
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