SUPERIOR PROTECTION AGAINST SHIV INFECTION BY SAME SITE DNA-PROTEIN CO-IMMUNIZATION

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Background: We compared immunogenicity and protective efficacy of an HIV vaccine comprised of DNA (*env* and *gag*) and Env proteins by co-administration of DNA and Protein in the same muscle or by separate administration of the DNA and Protein components in contralateral sites.

Methods: Female rhesus macaques (20 animals/group) were immunized with a 6-valent vaccine including DNA plasmids expressing membrane-anchored gp145 Env sequentially isolated from a HIV-1 infected individual (CH505). The DNA was delivered by IM injection followed by *in vivo* electroporation. The vaccine also included a gp120 Env protein component matching the sequences encoded by the plasmid DNA and adjuvanted in GLA-SE. The DNA and protein vaccine components were administered in the same anatomical sites ('Co-administration') or in contralateral sites ('Separate Administration') After 6 vaccinations in 4-month intervals, the macaques were challenged by weekly intravaginal exposures with low dose T/F tier-2 SHIV CH505 stock.

Results: Only macaques in the co-administration vaccine group were protected against SHIV CH505 acquisition, with a 67% risk reduction per exposure after 15 weekly IVAG challenges. Macaques in the co-administration group developed higher Env-specific humoral and cellular immune responses. Non-neutralizing Env antibodies, ADCC and antibodies binding to Fc-gamma Receptor IIIa were associated with decreased transmission risk. These data suggest that simultaneous recognition, processing and presentation of DNA + Env protein in the same draining lymph node play a critical role in the development of protective immunity.

Conclusions: Co-immunization of DNA+Protein in the same muscle is superior for inducing protective immune responses against repeated tier-2 SHIV challenge. The advantage of co-immunization vaccine regimens targeting immunogens to the same draining LN could also be beneficial to other vaccine modalities and other pathogens.

DNA + Protein Co-immunization Solves a Problem in HIV Vaccine Development: Induction of Durable Cellular and Humoral Immune Responses in Macaques

Vaccine	Ab response		Cellular response	
	magnitude	longevity	magnitude	longevity
protein	High; transient	NO		
DNA	moderate	yes	high	>8 yrs
DNA prime-protein boost	High; transient	moderate	high	yes
DNA + Protein co-immunization	**high**	**years**	**high**	**years**

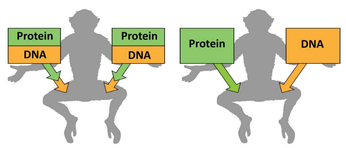
DNA + Protein co-immunization protocol

- Same site, same time delivery of DNA and protein
  - Different than standard DNA prime / protein boost
- Induction of durable humoral and cellular immune responses in macaques
- Provides protection from SIV infection and potent control of viremia and protection from AIDS development

Li, Vaccine 2013; Patel, PNAS 2013; Jalah, PLos One 2014; Valentin, Clin Immunol 2014; Vargas-Inchaustegui, Clin Immunol 2014; Singh, J Virol 2018

DNA+Protein Co-immunization Protocol

AIM: To compare immunogenicity and protective efficacy of two HIV DNA + protein vaccine delivery strategies in macaques

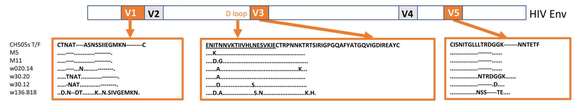


DNA + Protein 'Co-administration' | **DNA + Protein 'Separate Administration'**

Same vaccine, Same dose

Selection of Sequentially Isolated Clade C CH505 Env as Immunogens

- Env sequences were isolated from an African patient infected by clade C HIV (CH505) who developed CD4bs bNAbs *(Liao et al 2013 Nature)*
  - Sequential vaccination using 6 CH505 Env proteins to mimic *in vivo* antibody and Env co-evolution

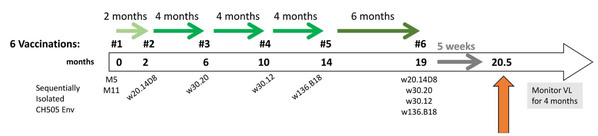


- Vaccine consists of matching DNA and protein

		Vaccine Adjuvant
Protein	6 CH505 gp120 Env	GLA-SE (TLR-4 agonist)
DNA	6 gp145 Env	Macaque IL-12
	SIV Gag	

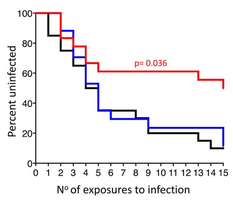
- Vaccine delivery: DNA delivery IM via electroporation (Cellectra 5P, Inovio)
  Protein delivery IM

Vaccination and Challenge Scheme



- 20 female macaques/group
- Extensive sampling of vaccinated macaques to allow in-depth analysis of immune responses

DNA + Protein Co-administration in the Same Anatomical Sites Protects from SHIV.CH505 infection



DNA + Protein Co-administration
9 of 18 vaccinees are protected
➢ 67% reduction in per exposure acquisition risk

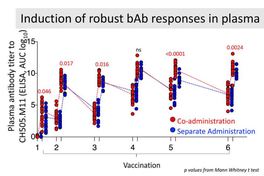
DNA + Protein Separate Administration
2 of 17 vaccinees remain uninfected

Control
2 of 20 animals remain uninfected

➢ Presence of the two vaccine components (DNA and protein) **in the same draining lymph nodes** plays a critical role in the development of protective immunity

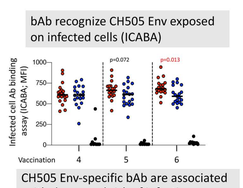
Vaccinees in the Co-administration Group Developed Higher anti-Env Antibody Responses

Induction of robust bAb responses in plasma

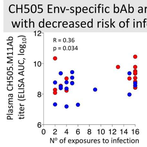


- Rapid induction of bAb responses
  - bAb can be detected after 1st vaccination
  - Sequential vaccinations boosted Ab levels with similar peak levels (4th to 6th vaccination)
- bAb have similar avidity among groups
- Similar linear peptide recognition among groups
- Similar levels of V2-specific Ab
- Higher levels of CD4 binding site Ab in the co-administration group
- Similar levels of mucosal dissemination of Ab

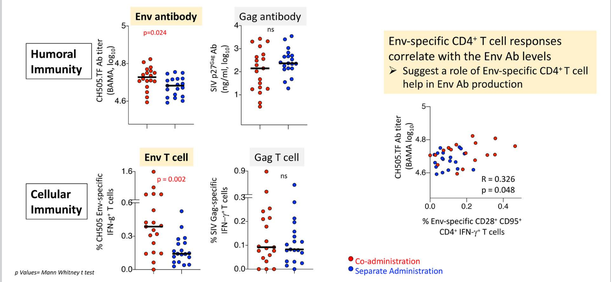
bAb recognize CH505 Env exposed on infected cells (ICABA)



CH505 Env-specific bAb are associated with decreased risk of infection



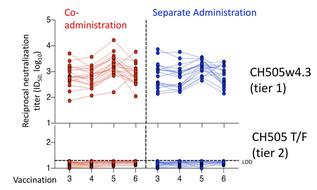
DNA + Protein Co-administration Vaccine Regimen Induces Higher Env Immune Responses



Env-specific CD4+ T cell responses correlate with the Env Ab levels

➢ Suggest a role of Env-specific CD4+ T cell help in Env Ab production

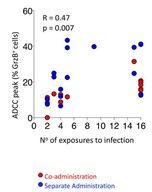
Induction of Robust NAb to CH505w4.3 (Tier 1) but Not to CH505 T/F (Tier 2)



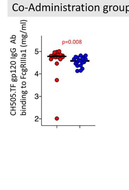
NAb to CH505.w4.3 did not correlate with decreased infection risk by SHIV.CH505 challenge

Non-neutralizing Antibodies Contribute to Reduced Risk of Infection

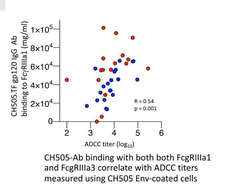
Peak ADCC activity is associated with resistance to infection



Higher CH505-specific Ab binding to FcγRIIIa in the Co-Administration group

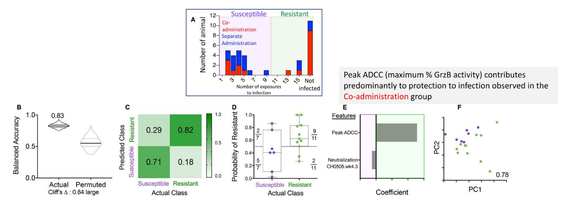


CH505-specific Ab binding to FcγRIIIa correlates with ADCC



CH505-Ab binding with both both FcgRIIIa1 and FcgRIIIa3 correlate with ADCC titers measured using CH505 Env-coated cells

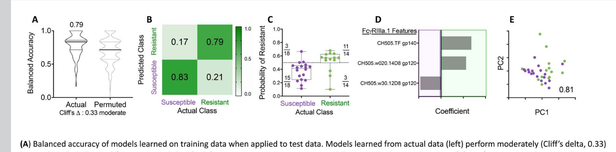
Multivariate Modeling of Correlates of Infection Risk Distinguishes Resistant Animals in the Co-administration Group



Peak ADCC (maximum % GrzB activity) contributes predominantly to protection to infection observed in the Co-administration group

(A) Histogram of challenge outcomes for all animals (indicating animals in the 2 vaccine groups) with sensitive and resistant animals defined as those infected (purple box) or not infected (green box) prior to the 10th challenge. **(B-F)** Models of infection resistance learned from immunogenicity data post 3rd immunization for the Co-administration group. A classifier was trained to use immunogenicity data to distinguish between animals based on challenge resistance. (**B**) Balanced accuracy of models learned on training data when applied to test data. Models learned from actual data (left) perform substantially (Cliff's delta, 0.84) better than those learned from permuted data (right). Robust performance and good overall average accuracy (83%) in the setting of repeated cross-validation was achieved. **(C)** Confusion matrix depicting the proportion of animals in each study arm predicted correctly/incorrectly. **(D)** Model confidence as defined by probability of belonging to the assigned class. **(E-F)** A final model was trained on the complete data and simplified to a sparse, two-feature model. (**E**) Coefficients of features in the simplified final model. **(F)** Principal component (PC) biplot of features contributing to the simplified final model. Simplified model accuracy is reported in inset. Resistant animals are indicated in green, susceptible animals in purple.

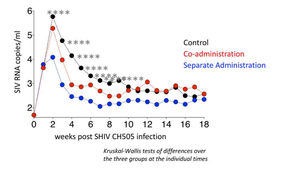
CH505 Ab Binding to FcγRIIIa Robustly Predicts Challenge Resistance

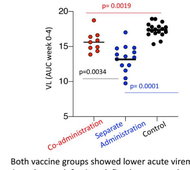


(A) Balanced accuracy of models learned on training data when applied to test data. Models learned from actual data (left) perform moderately (Cliff's delta, 0.33) better than those learned from permuted data (right). Average accuracy across cross-validation runs is reported in inset. **(B)** Confusion matrix depicting the proportion of animals in each study arm predicted correctly/incorrectly. (**C**) Model confidence as defined by the probability of belonging to the assigned class. (**D**) Coefficients of features in the final model. (**E**) Principal component (PC) biplot of features contributing to the final model. Final model accuracy is reported in inset. Resistant animals are indicated in green, susceptible animals in purple.

➢ Contribution of ADCC and the ability of CH505 Env-specific Ab to interact with FcγRIIIa are associated with and are predictive of reduced risk of infection

Both Vaccine Regimens Induce Immune Responses Able to Reduce Viremia



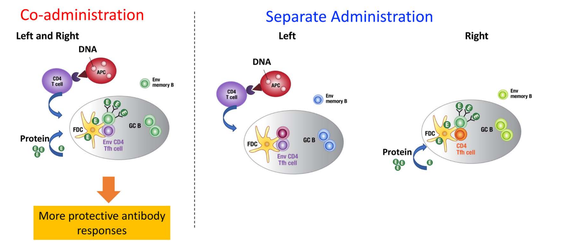


Both vaccine groups showed lower acute viremia during the first 4 weeks post-infection, defined as area-under-the-curve (AUC week 0-4), compared to controls .

- These data indicate that both vaccine regimens induced immune responses able to control viremia

Key difference between the 2 vaccine regimens is ability to protect from infection

Hypothesis: Distinct Priming of Adaptive Humoral Immune Responses in the Draining Lymph Nodes



Conclusions

Immune responses induced by an HIV DNA + protein combination vaccine co-administered simultaneously in the same anatomical site reduce infection risk upon pathogenic SHIV challenge, compared to separate delivery of the same DNA and protein components in contralateral sites.

- Co-administration of DNA (expressing Gag and Env) and Env protein vaccine components in the same muscle reduced SHIV infection risk by 67% per exposure.
- Macaques in the Co-administration group developed higher Env-specific humoral and cellular immune responses
- Non-neutralizing antibodies, ADCC activity and HIV Env antibody binding to FcγRIIIa served as a signature of reduced SHIV infection risk

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