CD40-targeted vaccine increases magnitude of HIV-Env specific responses

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BACKGROUND

The development of DC-targeted vaccines aims to increase the immunogenicity of proteins through improved antigen (Ag) delivery to specific Ag-presenting cells (APC) playing a key role in inducing and regulating immune memory. CD40 signaling is essential for T cell-dependent humoral responses and targeting vaccine antigen to CD40-expressing APC seems therefore an attractive option to promote anti-HIV antibody development.

To determine the impact of DC targeting on immune responses, we analyzed cellular changes at site of anti-CD40 vaccine injection.

METHODS

* **Model** : Cynomolgus macaque

* **Experimental groups**:
CD40 group: 6 animals immunized with HIVgp140 protein (ZM96) fused with anti-CD40 humanized monoclonal IgG4 Ab
IgG4 group: 6 animals immunized with HIVgp140 protein (ZM96) fused with IgG4 irrelevant control

* **Vaccine dose** : 200µg of vaccine protein + 1 mg PolyIC:LC (Hiltonol)

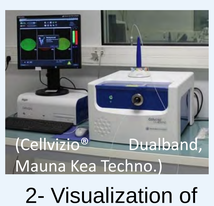
* **Immunization schedule** :
Homologous prime-boost strategy Week 0 - Week 4 - Month 9
Subcutaneous route

* **Immunogenicity assays:** Anti-gp140 specific immune responses were monitored over time by titration HIV-specific IgG binding antibody responses by ELISA, measurement of Neutralizing antibody responses against HIV-1 isolates (TZMbL assay), and follow-up of HIV env-specific T cell responses by IFN-g ELISPOT on PBMCs.

* ***In vivo* imaging**: Cellular trafficking was analyzed in injection site and draining lymph node by *in vivo* imaging after the boost



1-Identification of injection site and draning LN

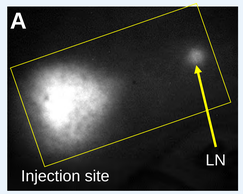


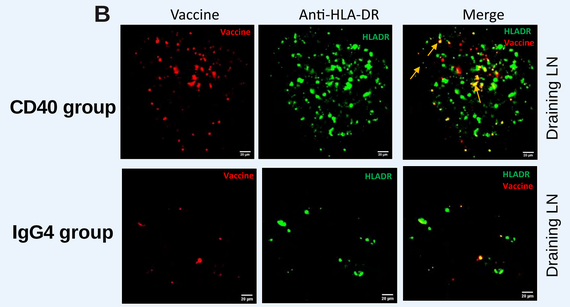
2- Visualization of vaccine-targeted cells and APCs

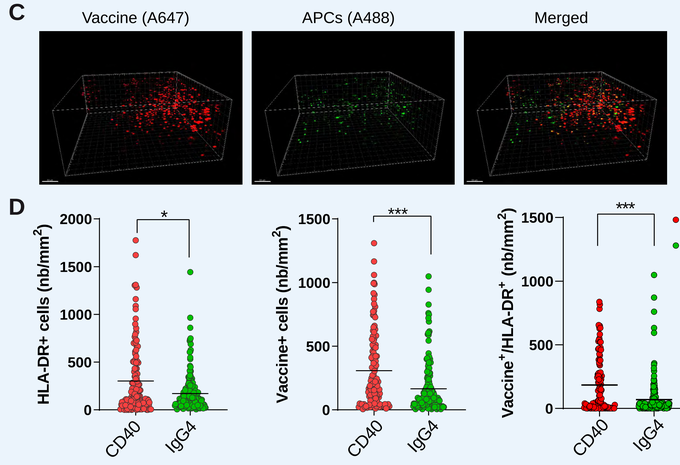


3- Depth penetration and 3D reconstruction

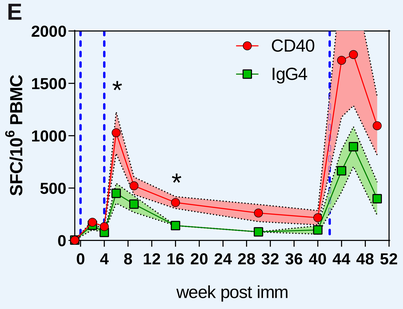
RESULTS

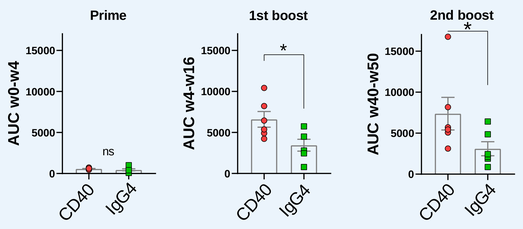




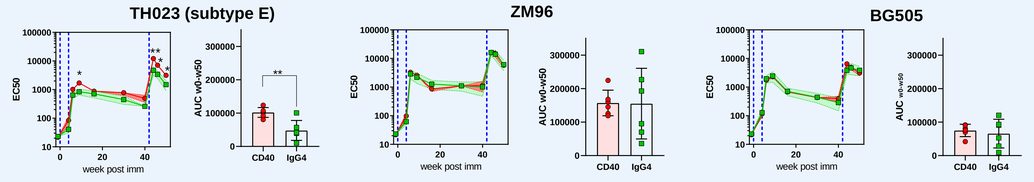


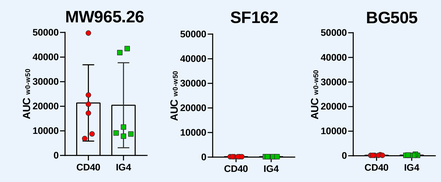
In vivo imaging analysis of draining LN. Lymph node of interest was identified by infrared imaging (**A**) and cell distribution was analyzed by *in vivo* confocal microscopy (**B**) and *in vivo* two-photon microscopy (**C**).
Quantification of vaccine positive cells and APC showed that anti-CD40 targeting increased the migration of antigen presenting cells and vaccine-targeted cells into the LN. (**D**) Quantification of vaccine targeted cells, anti-HLA-DR+ cells or double tagged cells within the draining LN. Mann-Whitney test was performed to compare experimental group; *p<0.05; ***p<0.001.





Targeting Env to CD40 increased the T cell responses against HIV Env peptides (**E**). Impact of CD40 targeting on T cell response was observed after the boost (**E & F**).
Responses were monitored by IFNg ELISPOT and expressed as number of SFC per millions of PBMC. Area Under the Curve (AUC) analyses of HIV env specific T cell responses after each immunization (**F**). Mann-Whitney test was performed to compare experimental group;*p<0.05; **p<0.005.





Anti-gp140 humoral response were evaluated by ELISA on serum samples. Immunizations induced specific antibody against Env proteins and is significantly higher against TH023 Env on animals which received the anti-CD40 vaccine (**G**). Specific neutralizing activity was induced against the homologous clade C isolate (**H**). Mann-Whitney test was performed to compare experimental group; *p<0.05; **p<0.005

CONCLUSIONS

Altogether these results highlighted that CD40 targeting increased the migration of antigen presenting cells and vaccine-targeted cells into the draining lymph node. We also demonstrated that the effects observed on the early immune events have an impact on the specific T and B cell responses, which is even more pronounced after a late boost. The safety and efficacy of the CD40-targeted vaccine justify further development for future human clinical trials.

ACKNOWLEDGMENT

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