Development of a Chimigen® Dendritic Cell Receptor-Targeted Multi-Antigen HIV Vaccine

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Abstract

Chimigen® Platform Technology has been used to design a novel dendritic cell (DC) receptor-targeted HIV vaccine that incorporates multiple antigens. This vaccine is capable of inducing antigen-specific cellular and humoral immune responses and has prophylactic/early intervention therapeutic applications.

Chimigen® Vaccines are chimeric recombinant fusion proteins of selected antigen(s) and specific xenotypic (murine) antibody fragments including the Fc region. These chimeric molecules bind to specific receptors on DCs and other antigen presenting cells for antigen uptake. They are processed through both proteasomal and endosomal pathways and presented to T cells through MHC class I and class II molecules, stimulating cellular and humoral immune responses against the chosen antigens.

The Chimigen® HIV Vaccine, containing the HIV-1 Gag, Env, Tat, Rev, Vpr and Vpu antigens, was expressed in Sf9 insect cells using a baculovirus expression system and purified. Immune responses to the vaccine were evaluated by *ex vivo* binding experiments and antigen presentation assays (APAs) using human peripheral blood mononuclear cell-derived DCs, T cells and B cells. Our results demonstrate that the Chimigen® HIV Vaccine binds to immature DCs in a dose-dependent manner. The APAs show that the vaccine induces $CD4^+$ and $CD8^+$ T cell activation and proliferation. Stimulation with vaccine-loaded DCs promoted increased production of IFN-γ and TNF-α from both $CD4^+$ and $CD8^+$ T cells. Furthermore, B cells stimulated with vaccine-loaded DCs were found to produce antigen-specific IgM antibodies. Taken together, our data suggests that this DC receptor-targeted HIV vaccine elicits humoral and cellular immune responses, and therefore, shows potential for development as a prophylactic/early intervention therapeutic vaccine against HIV infections.

Background

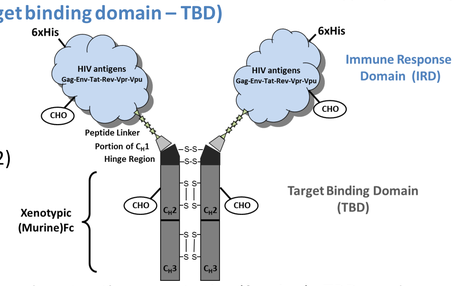
According to UNAIDS, an estimated 35 million people worldwide were living with HIV in 2012, and approximately 2 million people became newly infected. The current treatment for HIV infection, combination antiretroviral therapy (ART), can slow the progression of the disease; however, viral reservoirs persist in the body even with treatment. In addition, millions of infected individuals globally do not have access to ART. At present, there is no prophylactic or therapeutic vaccine available for HIV. Therefore, development of an effective and highly protective vaccine for HIV is a major global health priority. Our proprietary Chimigen® Platform Technology has been used to produce a HIV vaccine incorporating six HIV-1 antigens, and the results of our preliminary immunological evaluation are presented.

Unique characteristics of a Chimigen® Vaccine

Chimeric recombinant fusion protein with properties of both **antigen(s) (immune response domain - IRD)** and the Fc fragment of a xenotypic (murine) monoclonal **antibody (target binding domain – TBD)**

Versatile platform; can incorporate any relevant antigen

Facilitates binding to FcγRII (CD32) and macrophage mannose receptors (CD206) on dendritic cells (DCs)



Increased immunogenicity due to the xenotypic (foreign) TBD and non-mammalian glycosylation (depicted "CHO") from expression in insect cells

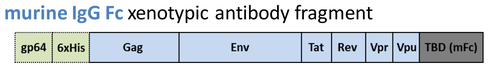
Antigen presentation *via* MHC class I and class II pathways; induces both **cellular and humoral immune responses**

Potential use for development of both prophylactic and therapeutic vaccines

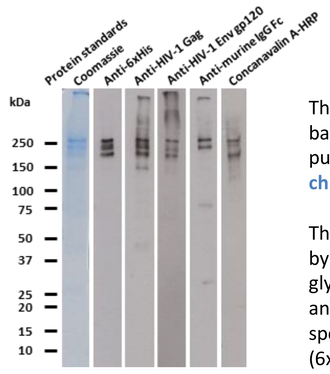
No added adjuvant and effective at low doses

Chimigen® HIV Vaccine design

N-terminal **gp64** signal sequence and **6xHis** tag
Complete HIV antigens: **Gag** (structural proteins), **Env** (envelope spike gp120, gp41)
Tat, Rev, Vpr, Vpu (regulatory/accessory proteins)
C-terminal **murine IgG Fc** xenotypic antibody fragment



The Chimigen® HIV Vaccine was cloned, expressed, and purified



The HIV vaccine DNA sequence was **codon-optimized** for expression in insect cells and synthesized.

The vaccine was cloned, produced using baculovirus-infected Sf9 insect cells, and purified by **Protein A and nickel affinity chromatography**.

The purified HIV vaccine was characterized by SDS-PAGE (Coomassie staining), glycostaining (Concanavalin A-HRP staining), and Western blot analysis using antibodies specific to different regions of the vaccine (6xHis, Gag, Env gp120, and IgG Fc).

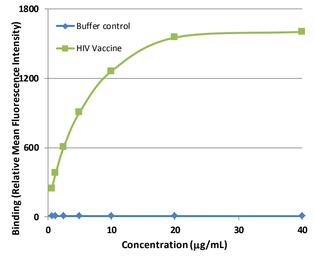
The N-terminus, HIV antigens, and C-terminus of the purified Chimigen® HIV Vaccine are intact, and the vaccine is glycosylated.

The Chimigen® HIV Vaccine binds to immature dendritic cells

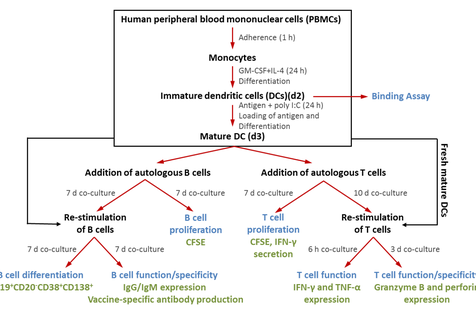
Binding Assay:
PBMC-derived immature DCs were incubated with the vaccine (1-40 µg/mL) or buffer for 1 h. The relative amount of bound vaccine was detected by flow cytometry following labeling with biotinylated anti-mouse IgG1 mAb and streptavidin-PE-Cy5.

The Chimigen® HIV Vaccine binds to cultured immature DCs in a saturable and dose-dependent manner.

Binding of the Chimigen® HIV Vaccine to 48 hr Cultured Immature DCs



Antigen presentation assay



Antigen presentation assays (APAs) measure the **immune response of naïve T cells or B cells to antigen presented by DCs**, *ex vivo.*

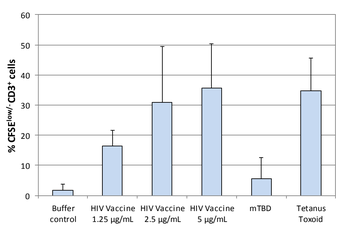
APAs **quantify functional T cell or B cell immune responses** and the ability of antigen-loaded mature DCs to **expand** antigen-specific T cell or B cell clones.

Chimigen® HIV Vaccine induces proliferation of $CD3^+$ T cells

APA - T Cell Proliferation Assay:
CFSE-labeled T cells were stimulated with antigen-loaded mature DCs (buffer control, vaccine, mTBD and tetanus toxoid control proteins) and T cell proliferation was measured in triplicate by the loss of CFSE dye using flow cytometry.

The HIV vaccine induces $CD3^+$ T cell activation and expansion *ex vivo* following the first exposure of naïve T cells to vaccine-pulsed mature DCs.

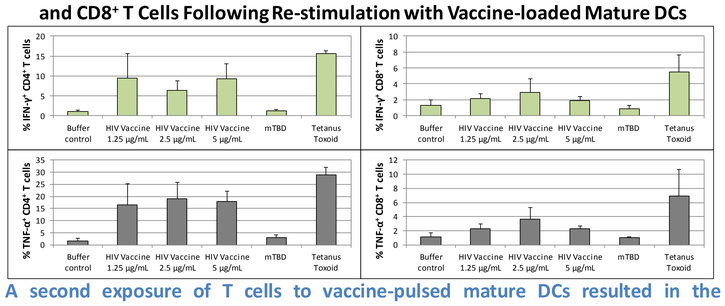
Induction of T Cell Proliferation in $CD3^+$ ($CD4^+$ and $CD8^+$) T Cells by the Chimigen® HIV Vaccine



Vaccine induces IFN-γ and TNF-α production in $CD4^+$ & $CD8^+$ T cells

APA - Th1 Cytokine Expression (IFN-γ and TNF-α):
The percentage of IFN-γ- and TNF-α-expressing $CD4^+$ and $CD8^+$ T cells was quantified by intracellular cytokine staining and flow cytometry following **re-stimulation with vaccine/ antigen-loaded mature DCs.**

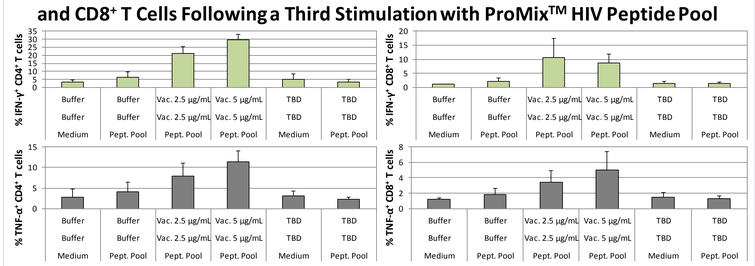
Induction of IFN-γ and TNF-α Expression by the Chimigen® HIV Vaccine in $CD4^+$ and $CD8^+$ T Cells Following Re-stimulation with Vaccine-loaded Mature DCs



A second exposure of T cells to vaccine-pulsed mature DCs resulted in the enhanced expression of IFN-γ and TNF-α in both $CD4^+$ and $CD8^+$ T cells.

APA - T Cell Recall Response (specificity of the immune response):
IFN-γ and TNF-α production in buffer-, vaccine-, and mTBD-primed T cells was measured following **re-stimulation with a pool of HIV-1 peptides** (Gag, Env).

Induction of IFN-γ and TNF-α Expression by the Chimigen® HIV Vaccine in $CD4^+$ and $CD8^+$ T Cells Following a Third Stimulation with ProMix™ HIV Peptide Pool

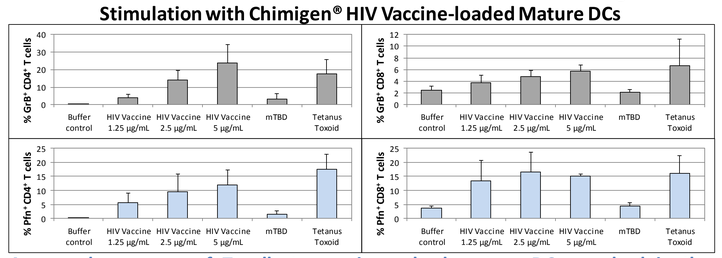


Vaccine-primed T cells re-stimulated with a HIV peptide pool showed an increase in $CD4^+$ and $CD8^+$ T cell IFN-γ and TNF-α production.

Vaccine induces GrB and Pfn expression in $CD4^+$ & $CD8^+$ T cells

APA – Granzyme B and Perforin Expression (cytotoxic T cell response):

Induction of GrB and Pfn Expression in $CD4^+$ and $CD8^+$ T Cells Following a Second Stimulation with Chimigen® HIV Vaccine-loaded Mature DCs



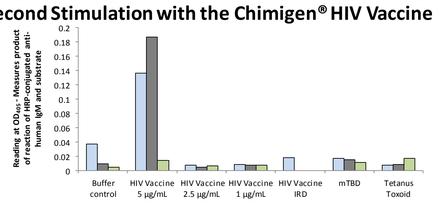
A second exposure of T cells to vaccine-pulsed mature DCs resulted in the enhanced expression of GrB and Pfn in both $CD4^+$ and $CD8^+$ T cells.
The Chimigen® HIV Vaccine induces specific functional T cell responses *ex vivo*.

Vaccine induces production of antigen-specific IgM Abs in B cells

APA - B Cell Function:
Anti-HIV Vaccine IgM antibody was detected in the culture medium by ELISA.

The Chimigen® HIV Vaccine induces specific functional B cell responses *ex vivo*.

Antigen-specific IgM Expression in B Cells Following a Second Stimulation with the Chimigen® HIV Vaccine



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