Chimigen® HCV Prophylactic/Therapeutic Vaccine: Immune Responses

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ABSTRACT

Chimigen® Vaccines are a new class of chimeric molecules with functional attributes of both antigen(s) and a xenotypic monoclonal antibody. These molecules target the host immune system using specific receptors on dendritic cells (DCs) and are processed for antigen presentation through both MHC class I and class II pathways to elicit cellular and humoral immune responses against the selected antigens. Chimigen® HCV Prophylactic/Therapeutic Vaccine, a fusion protein of HCV antigens (E1-E2-NS5A) and a murine Fc fragment, has been produced in insect cells, purified and characterized.

The immune responses to the vaccine were evaluated *ex vivo* using human peripheral blood mononuclear cell (PBMC)-derived DC/T cells from uninfected donors and chronically HCV-infected patients using an antigen presentation assay (APA). PBMCs were stimulated with buffer, vaccine, HCV antigen, or the xenotypic Fc fragment and then re-stimulated with vaccine-loaded DCs or overlapping peptides of HCV antigens. In cells derived from both healthy donors and patients chronically infected with HCV, stimulation with the vaccine or the overlapping peptides induced a marked increase in the percentage of CD8+ and CD4+ T cells producing IFN-γ and TNF-α. Vaccine-activation of PBMCs from HCV-infected patients stimulated granzyme B production by $CD4^+CD25^-$ responder T cells (Tresp) which leads to apoptosis of $CD4^+CD25^+$ regulatory T cells (Tregs). B cells were isolated from PBMCs and co-cultured with vaccine-loaded DCs. The primed B cells were re-stimulated with vaccine-loaded DCs, which resulted in the production of HCV-vaccine-specific IgM and IgG antibodies detectable in culture supernatants. The expansion of HCV-antigen specific CD4+ and CD8+ T cells, production of Tresp cells and production of specific antibodies show the potential of the Chimigen® HCV Vaccine for use as a prophylactic/therapeutic vaccine for hepatitis C.

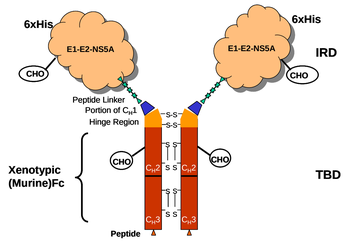
INTRODUCTION

Great advances have been made in recent years in the development of antiviral treatments to cure HCV infections. The prime examples of these include Sofosbuvir, a nucleotide analog inhibitor of NS5B polymerase, and Daclatasvir, an NS5A complex formation inhibitor. The major limitation of these treatments is the extremely high cost of therapy, especially for patients in developing countries An effective prophylactic/therapeutic vaccine to prevent or cure HCV infections is still a major unmet medical need.

Chimigen® HCV E1-E2-NS5A Vaccine is designed to prevent infection as well as to resolve chronic HCV infection by targeting the selected antigens to antigen presenting cells, especially DCs through specific receptors, to induce both humoral and cellular immune responses. Among the HCV antigens, NS5A contains several $CD8^+$ CTL and $CD4^+$ T helper epitopes and has been shown to be important for the assembly of the cytoplasmic membrane-bound complex for HCV replication. Therefore, the NS5A antigen provides a valid target for a therapeutic vaccine. The envelope glycoproteins, E1 and E2, are essential for virus binding and uptake by target cells. Thus, inclusion of the E1 and E2 antigens in a prophylactic vaccine is essential for production of neutralizing antibodies to block virus entry.

In this study, we have evaluated the ability of the vaccine to elicit antigen-specific T cell and B cell responses in PBMCs derived from uninfected healthy donors and from patients with chronic HCV infection.

Chimigen® HCV *E1-E2-NS5A* Recombinant Molecule



Unique Characteristics of the Chimigen® Vaccine

- **Fusion protein comprised of antigen (Immune Response Domain – IRD) and the Fc portion of a xenotypic monoclonal antibody (Target Binding Domain - TBD)**
- **Adaptable platform; can incorporate any relevant antigen**
- **Increased immunogenicity due to the xenotypic nature of the TBD and expression in insect cells which imparts non-mammalian glycosylation**
- **No added adjuvant**
- **Effective at very low doses (µg)**
- **The TBD facilitates binding of Chimigen® Vaccines to Fcγ receptors on DCs**
- **The glycosylation facilitates the binding to C-type lectin receptors on antigen presenting cells, especially DCs**
- **Antigen presentation *via* MHC class I and class II pathways**
- **Generates cellular and humoral immune responses, defined by IRD**

RESULTS

Chimigen® HCV Vaccine has been Expressed, Purified and Characterized

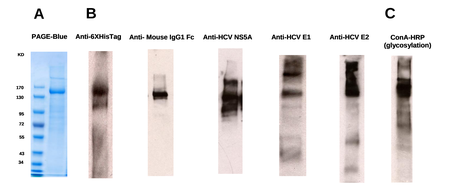


Figure 1. Purified Chimigen® HCV E1-E2-NS5A Vaccine. The fusion protein was purified using HisTrap FF and characterized by PAGE, Western blots using antibodies specific to different regions of the vaccine and glycostaining. **(A)** 7.5% SDS PAGE PageBlue-stained, **(B)** Western blots using antibodies specific to 6xHis, IRD domain (anti-HCV antigens) and TBD (anti-Fc),**(C)** Glycosylation detected by staining with ConA conjugated to HRP.

Chimigen® HCV Vaccine Binds to Immature DCs

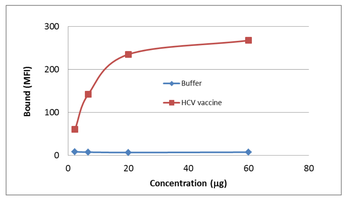
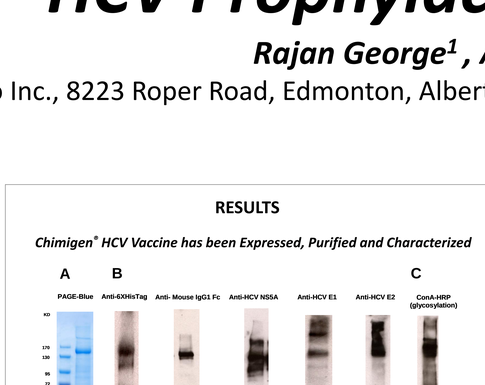


Figure 2. PBMC-derived immature DCs were incubated for 1 hr at 4°C with 1-60 µg/ml Chimigen® E1-E2-NS5A Vaccine. Bound protein was detected by flow cytometry following labeling with biotinylated anti-mouse IgG mAb and SA-PE-Cy5. The vaccine binds to FcγRII and C-type Lectin receptors (Data not presented).

Assessment of T Cell and B Cell Responses to Chimigen® HCV Vaccine – Antigen Presentation Assay



Chimigen® HCV Vaccine Induces Proliferation of $CD8^+$ and $CD4^+$ T cells

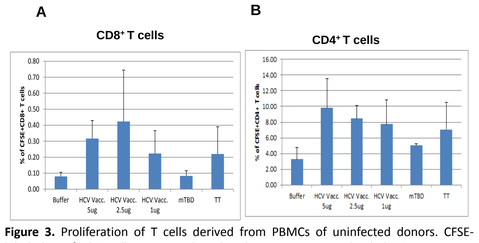


Figure 3. Proliferation of T cells derived from PBMCs of uninfected donors. CFSE-labeled purified T cells were incubated with mature, vaccine-loaded DCs or vaccine component-loaded DCs for 9 days. Cells were harvested and the percentage of $CFSE^{low/-}$ $CD8^+$ **(A)** and $CD4^+$ **(B)** T cells was determined by flow cytometry.

Chimigen® HCV Vaccine Induces IFN-γ Secretion by T Cells After a Single Stimulation

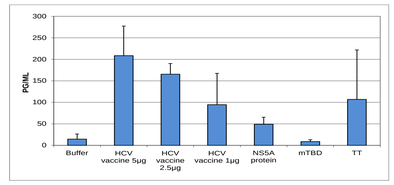


Figure 4. IFN-γ secretion by T cells derived from uninfected donor PBMCs. T cells were incubated with mature, vaccine-loaded DCs for 9 days. On day 9, an aliquot of culture medium was collected and used for an IFN-γ ELISA.

Chimigen® HCV Vaccine Induces IFN-γ and TNF-α Production in $CD4^+$ and $CD8^+$ T Cells

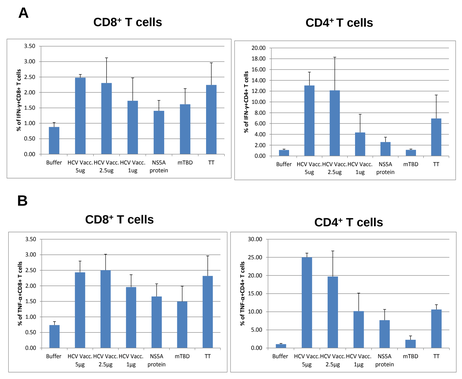


Figure 5. Th1 cytokine production by T cells derived from PBMCs of uninfected donors. Purified T cells were incubated with mature, vaccine-loaded DCs for 9 days. T cells were then re-stimulated with mature, vaccine-loaded DCs for 6 hours. Tetanus toxoid (TT) is a positive control. The percentage of T cells producing IFN-γ **(A)** or TNF-α **(B)** was determined by intracellular cytokine staining and flow cytometry.

Chimigen® HCV Vaccine Induces Granzyme B Production in $CD8^+$ and $CD4^+$ T Cells

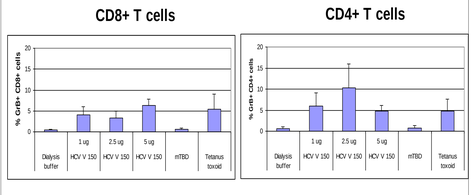


Figure 6. Granzyme B production by T cells derived from PBMCs of uninfected donors. Purified T cells were incubated with mature, vaccine-loaded DCs for 9 days. T cells were then restimulated with mature, vaccine-loaded DCs for 3 days. Cells were harvested and the percentage of GrB^+CD8^+ and GrB^+CD4^+ T cells was determined by intracellular cytokine staining and flow cytometry.

Chimigen® HCV Vaccine Increased Intracellular Th1 Cytokine Expression in $CD8^+$ and $CD4^+$ T cells from Chronically Infected Donors

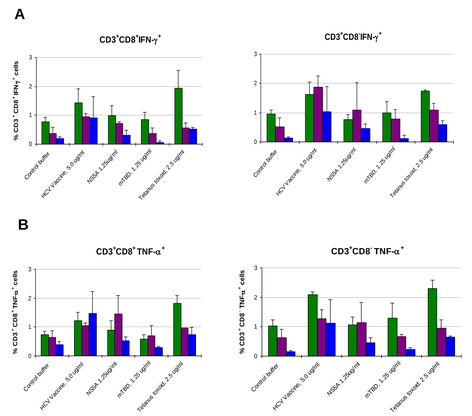


Figure 7. IFN-γ and TNF-α production by T cells (PBMCs derived from chronically HCV-infected donors). PBMCs were incubated with the vaccine for 9 days. PBMCs were then re-stimulated with mature, vaccine-loaded DCs for 6 hours. The percentage of $CD8^+$ or $CD4^+$ T cells producing IFN-γ **(A)** or TNF-α **(B)** was determined by intracellular cytokine staining and flow cytometry. Each colour represents an individual donor.

Chimigen® HCV Vaccine Induces IFN-γ Secretion by PBMCs Derived From Chronically Infected Donors

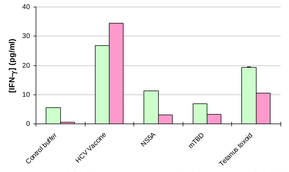


Figure 8. IFN-γ secretion by PBMCs derived from HCV carriers. PBMCs were incubated with 5 µg/ml of vaccine for 9 days. After 9 days, an aliquot of culture media was used for an IFN-γ ELISA. Each colour represents an individual donor.

Chimigen® HCV Vaccine Induces HCV Antigen-specific $CD8^+$ and $CD4^+$ T Cell Intracellular IFN-γ Expression in Chronically Infected Donors

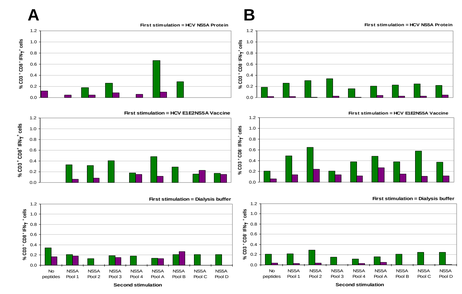


Figure 9. IFN-γ production by vaccine-treated T cells (PBMCs derived from chronically HCV-infected donors) that were re-stimulated with sets of overlapping peptides covering HCV NS5A protein. PBMCs were incubated with vaccine for 9 days. Pools of overlapping peptides were added to the PBMC cultures on day 9 and the cultures were incubated for 6 hours. The percentage of $CD8^+$ **(A)** and $CD4^+$ **(B)** T cells producing IFN-γ was determined by intracellular cytokine staining and flow cytometry. Each colour represents an individual donor.

Chimigen® HCV Vaccine Induces Production of Tresp cells and Triggers Treg Cell Apoptosis in Chronic HCV Carrier

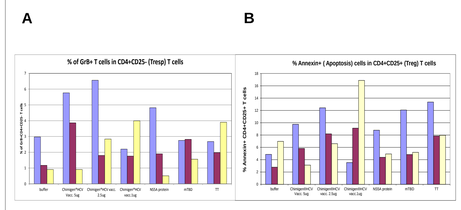


Figure 10. Induction of functional Tresp/Treg responses by the Chimigen® HCV Vaccine. PBMCs derived from an HCV carrier (#25) were stimulated with the vaccine for 7 days and then labeled with anti-human CD4-(PerCP)and CD25-(APC) antibodies. Following surface staining, the cells were fixed, permeabilized, and then incubated with anti-human GrB-FITC **(A)**. For monitoring apoptotic cells in Tregs, the PBMCs were stimulated with vaccine for 7 days and then labeled with anti-human CD4 and CD25 antibodies and Annexin V-(FITC)**(B)**. The cells were fixed after surface staining and acquired using the FACSCalibur. Each colour bar represents an single well of a triplex.

Chimigen® HCV Vaccine Induces Antigen-specific IgM/IgG Ab Production

Figure 11. Detection of antigen-specific IgM **(A)** and IgG **(B)** production in culture supernatants. Supernatants were collected on day 7 after the second stimulation with vaccine. The ELISA plate was coated with Chimigen®HCV Vaccine at 0.1µg/well. After 1 h incubation at 37°C, the plate was washed and HRP conjugated rabbit anti-human IgG or IgM antibody was added. After 1h incubation, the plate was washed and the colour was developed. Each colour bar represents a single well of a triplex.

CONCLUSIONS

- **The Chimigen® HCV E1-E2-NS5A Vaccine was produced in Sf9 insect cells, purified and characterized**
- **The vaccine binds to specific receptors on immature DCs**
- **The vaccine induces proliferation of T cells from uninfected donors**
- **Both T cells from uninfected donors and PBMCs from chronically HCV-infected donors secrete IFN-γ after stimulation with vaccine**
- **The vaccine induces production of the Th1 cytokines IFN-γ and TNF-α by $CD8^+$ and $CD4^+$ T cells (uninfected and chronically HCV-infected donors)**
- **Vaccine stimulation results in production of Tresp cells and killed Treg cells in PBMCs isolated from a chronically infected donor**
- **Vaccine stimulation of B cells *in vitro* induces antigen-specific IgM and IgG antibody production**
- **The Th1 T cell and B cell immune responses demonstrated in the *ex vivo* assays suggest that the Chimigen® HCV E1-E2-NS5A Vaccine has potential use as a prophylactic/therapeutic vaccine for the prevention/ treatment of HCV infections**

ACKNOWLEDGEMENTS

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