Immune Responses to a Novel Chimigen® HCV Prophylactic/Therapeutic Vaccine

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ABSTRACT

Chimigen® Vaccines are a new class of chimeric molecules with functional attributes of both an antigen and a xenotypic monoclonal antibody. These molecules target the host immune system using specific receptors on dendritic cells (DCs) and are processed for presentation to T cells through both MHC class I and class II pathways to elicit cellular and humoral immune responses against specific viral 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 using human peripheral blood mononuclear cell (PBMC)-derived DC/T cells, in an antigen presentation assay (APA), ex vivo. The immune responses to the vaccine were assessed in T cells derived from patients with chronic HCV infection and uninfected healthy donors. PBMCs were stimulated *ex vivo* with buffer, vaccine, HCV antigen, or the xenotypic Fc fragment and then re-stimulated with vaccine-loaded DCs or HCV antigen overlapping peptides. In cells derived from both healthy donors and chronically infected patients, a single stimulation with the vaccine resulted in increased IFN-γ secretion as compared to treatment with vaccine components. Re-stimulation with vaccine or the overlapping peptides induced a marked increase in the percentage of $CD8^+$ and $CD4^+$ T cells producing IFN-γ and TNF-α. The induction of the expansion of HCV-antigen specific $CD4^+$ and $CD8^+$T cells from chronically infected patients shows the potential of Chimigen® HCV Vaccine for the development as a prophylactic/ therapeutic vaccine for HCV infections.

INTRODUCTION

More than 170 million people worldwide are chronically infected by the Hepatitis C virus (HCV). New infections are occurring at the rate of 2 -3 million annually, and more than 3 million patients in the U.S. and Canada may have the disease. Infected patients can remain asymptomatic for decades before developing liver cirrhosis and/or hepatocellular carcinoma. Unlike HBV, there is currently no vaccine available to prevent HCV infection. HCV patients currently receive a combination of interferon-α and ribavirin. However, this combination is expensive, has substantial side effects and is effective in approximately 50% of a select group of patients. Telaprevir and Boceprevir, the two recently approved direct acting HCV antiviral molecules inhibit viral replication by blocking activity of the HCV NS3 serine protease. Used in combination with the current therapy, these two new antivirals are expected to cure between 60-70% of the HCV infections. Even with these new developments in HCV therapeutics, there is still need for an effective therapeutic vaccine for the treatment of patients chronically infected with HCV. Moreover, an effective prophylactic vaccine for HCV is a major unmet medical need. Previous attempts to develop HCV therapeutic as well as prophylactic vaccines resulted in very limited success. New approaches in developing HCV vaccines are necessary.

In chronic states of HCV infection, the virus may escape attack by the host immune system because the viral antigens are recognized as "self," a consequence of the lack of recognition by the antigen-presenting cells. On exposure to HCV, individuals who develop a self-limited infection mount a strong and multi-specific CD4+ (helper) and CD8+ (cytotoxic) T cell response to the virus. In contrast, individuals who mount a weak and narrowly focused T cell response are unable to clear the virus and become chronic HCV carriers.

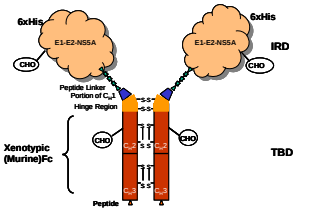
It is widely accepted that the host immune system plays a key role in the outcome of HCV infection. Failure of patients with HCV infection to initiate and sustain a strong Th1 response may be due to lack of proper presentation of the appropriate viral antigen to host immune cells. The success in eliminating the virus results from the manner in which the antigen is processed and presented by the antigen presenting cells (APCs) and the involvement of cytotoxic T cells. The generation of a humoral response is essential to prevent infection whereas a CTL response is critical for the elimination of the virus-infected cells and thus in the resolution of a chronic infection.

A novel class of chimeric molecules (Chimigen® Vaccines) with attributes of antigen(s) and a xenotypic monoclonal antibody have been designed and produced. These chimeric fusion proteins are designed to bind to specific receptors on dendritic cells, directing the antigen to the appropriate cellular compartments for antigen processing and presentation which result in the generation of both cellular and humoral immune responses.

The Chimigen® HCV E1-E2-NS5A Prophylactic/Therapeutic Vaccine, a fusion protein of HCV antigens and a murine Fc fragment, has been cloned, expressed and purified. Chimigen® HCV Vaccine is designed to induce strong host immune responses to prevent infection as well as to resolve chronic HCV infection. Among the HCV antigens, NS5A, a nonstructural protein, contains several $CD8^+$ CTL and $CD4^+$ T helper epitopes. NS5A has been shown to be involved in the regulation of viral replication and host cell interactions, therefore, provides a valid target for a therapeutic vaccine. The envelope glycoproteins, E1 and E2, are essential for virus binding and uptake by target cells and therefore the production of neutralizing antibodies to block virus entry is essential for a prophylactic vaccine.

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 patients with chronic HCV infection and uninfected healthy donors.

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 high (pauci) mannose 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

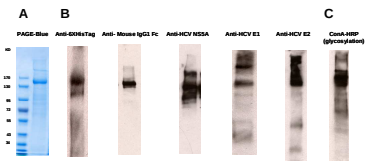


Figure 1. Purified Chimigen® HCV E1-E2-NS5A Vaccine. The fusion protein was purified using HisTrap FF and characterized by PAGE, Western blots using antibodies specific to different regions of the vaccine and glycostaining. **(A)** 7.5% SDS PAGE PageBlue-stained, **(B)** Western blots using antibodies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

Chimigen® HCV Vaccine Binds to Immature DCs

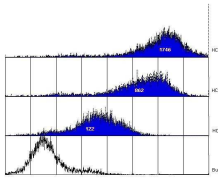
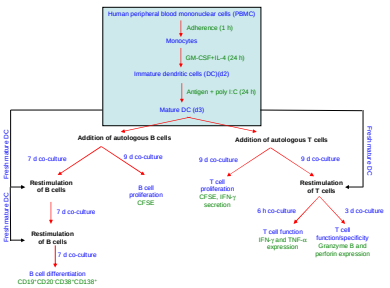


Figure 2. PBMC-derived immature DCs were incubated for 1 hr at 4°C with 1-20 μg/ml Chimigen® E1-E2-NS5A Vaccine. Bound protein was detected by flow cytometry following labeling with biotinylated anti-mouse IgG mAb and SA-PE-Cy5.

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



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

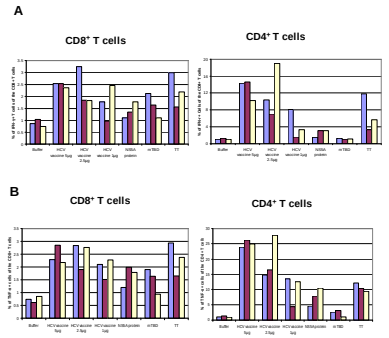


Figure 3. Th1 cytokine production by T cells derived from PBMCs of un-infected donors. Purified T cells were incubated with mature, vaccine-loaded DCs for 9 days. T cells were then re-stimulated with mature, vaccine-loaded DCs for 6 hours. Tetanus toxoid (TT) is a positive control. The percentage of T cells producing IFN-γ **(A)** or TNF-α **(B)** was determined by intracellular cytokine staining and flow cytometry. Each coloured bar represents one well from a triplicate.

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

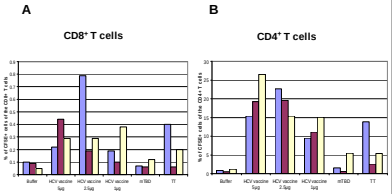


Figure 4. Proliferation of T cells derived from PBMCs of uninfected donors. CFSE-labeled purified T cells were incubated with mature, vaccine-loaded DCs or vaccine components-loaded DCs for 9 days. Cells were harvested and the percentage of **(A)** $CFSE^{low/-}$ $CD8^+$ and **(B)** $CD4^+$ T cells was determined by flow cytometry. Each coloured bar represents one well from a triplicate

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

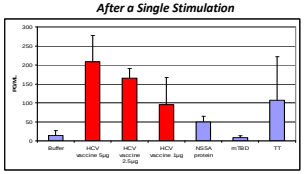


Figure 5. IFN-γ secretion by T cells derived from uninfected donor PBMCs. T cells were incubated with mature, vaccine-loaded DCs for 9 days. After 9 days, an aliquot of culture media was collected and used for an IFN-γ ELISA.

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

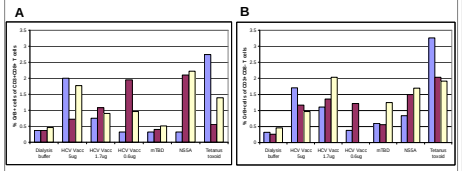


Figure 6. Granzyme B production by T cells derived from PBMCs of uninfected donors. Purified T cells were incubated with mature, vaccine-loaded DCs for 9 days. T cells were then restimulated with mature, vaccine-loaded DCs for 3 days. Cells were harvested and the percentage of **(A)** GrB$^+$CD8$^+$ and **(B)** GrB$^+$CD4$^+$ T cells was determined by intracellular cytokine staining and flow cytometry. Each coloured bar represents one well from a triplicate.

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

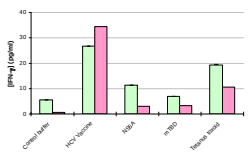


Figure 7. IFN-γ secretion by PBMCs derived from HCV carriers. PBMCs were incubated with 5 μg/ml of vaccine for 9 days. After 9 days, an aliquot of culture media was used for an IFN-γ ELISA. Each colour bar represents an individual donor.

Chimigen® HCV Vaccine Presentation by Mature DCs Results in Increased Intracellular Th1 Cytokine Expression in $CD8^+$ and $CD4^+$ T cells

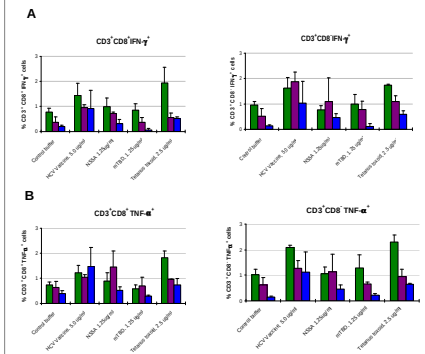


Figure 8. IFN-γ and TNF-α production by T cells (PBMCs derived from chronically HCV-infected donors). PBMCs were incubated with the vaccine for 9 days. PBMCs were then re-stimulated with mature, vaccine-loaded DCs for 6 hours. The percentage of $CD8^+$ or $CD4^+$ T cells producing IFN-γ **(A)** or TNF-α **(B)** was determined by intracellular cytokine staining and flow cytometry. Each coloured bar represents an individual donor.

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

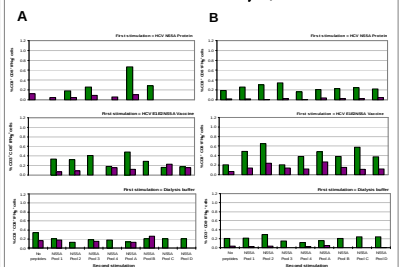


Figure 9. IFN-γ production by vaccine-treated T cells (PBMCs derived from chronically HCV-infected donors) that were re-stimulated with sets of overlapping peptides covering HCV NS5A protein. PBMCs were incubated with vaccine for 9 days. Pools of overlapping peptides were added to the PBMC cultures on 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 bar represents an individual donor.

Chimigen® HCV Vaccine Induces Proliferation of B Cells

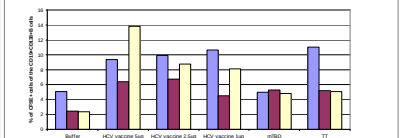


Figure 10. Proliferation of B cells (PBMCs derived from uninfected donors). Purified B cells were CFSE-labeled and incubated with mature DCs loaded with either the vaccine or vaccine components for 9 days. Cells were harvested and the percentage of CFSE low/- CD19$^+$CD138$^+$ was determined by flow cytometry. Each colour bar represents one well from a triplicate assay.

Chimigen® HCV Vaccine Induces Differentiation of B Cells from Uninfected Donors to Mature B Cells

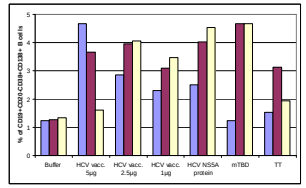


Figure 11. Differentiation of B cells. Purified B cells from uninfected donors were incubated with mature, vaccine-loaded DCs for 7 days. Cells were re-stimulated two times at 7 day intervals. Cells were harvested 7 days after the last stimulation and the percentage of CD19$^+$CD20 CD38$^+$CD138$^+$ was determined by flow cytometry. Each colour bar represents one well from a triplicate assay.

CONCLUSIONS

- The Chimigen® HCV E1-E2-NS5A Vaccine has been cloned, expressed and purified
- The vaccine binds to immature DCs
- The vaccine induces proliferation of T cells from uninfected and chronically HCV-infected donors
- Vaccine-treated PBMCs from uninfected and chronically HCV-infected donors secrete IFN-γ
- 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 HCV antigen-specific T cell expansion from PBMCs isolated from chronically infected donors
- The Vaccine induces proliferation and maturation of B cells
- The Th1 T cell and B cell immune responses demonstrated in the *ex vivo* assays suggests that the Chimigen® HCV E1-E2-NS5A Vaccine has potential use as a prophylactic/therapeutic vaccine for the prevention/ treatment of HCV infections

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