Chimigen® HCV Therapeutic Vaccine Induces a Robust Immune Response in Chronic HCV Carrier Peripheral Blood Mononuclear Cells, *ex vivo*

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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 Therapeutic Vaccine, a fusion protein of HCV antigen and a murine Fc fragment, has been produced in insect cells, purified and characterized.

Persistent exposure to viral antigens during chronic HCV infection may lead to functional impairment or deletion of virus-specific T cells. The goal of this study was to assess T cell responses to the vaccine in T cells derived from patients with chronic HCV infection and uninfected healthy donors. Peripheral blood mononuclear cells (PBMC) were stimulated *ex vivo* with buffer, vaccine, HCV antigen, or the xenotypic Fc fragment. 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-loaded DCs induced a marked increase in the percentage of $CD8^+$ and $CD4^+$ T cells producing IFN-γ and TNF-α. The expansion of HCV-antigen specific $CD4^+$ and $CD8^+$ T cells in PBMC from chronically infected patients shows the potential for the development of a therapeutic vaccine for treatment of chronic HCV infections.

INTRODUCTION

Hepatitis C virus (HCV) has chronically infected approximately 170 million people worldwide. 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. At present there is no vaccine 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. No approved therapeutic vaccine for the treatment of patients chronically infected with HCV is currently available. There are several therapeutic vaccine candidates under development by different companies using mostly DNA and viral vectors. These vaccines are in preclinical or early clinical testing. Therapeutic vaccines to treat chronic HCV infections are major unmet medical needs.

In chronic states of HCV infections, the virus may escape the attack by the host immune system as the viral antigens are recognized as "self," a consequence of the lack of recognition by the antigen-presenting cells. Individuals that become exposed to HCV mount a strong and broad CD4+ (regulatory) and CD8+ (cytotoxic) T cell response to the virus. These individuals develop only a self-limited infection. In contrast, about 50% of adults exposed to HCV mount a weak or undetectable and narrowly focused T cell response. As these individuals are unable to clear the virus they become chronic HCV carriers.

It is widely accepted that host immune system plays a key role in the outcome of an HCV infection. The failure of HCV patients to initiate, maintain, and sustain a strong Th1 response may be due to the lack of proper presentation of the appropriate viral antigen(s) to the host immune system. The success in eliminating the virus may also result from the manner in which the antigen is processed and presented by the antigen presenting cells (APCs) and the involvement of regulatory and cytotoxic T cells. The generation of a CTL response is critical in the elimination of the virus-infected cells and thus in the resolution of the infection.

Therapeutic vaccines that induce strong host immune responses may help to resolve chronic HCV infection. Among the HCV antigens, HCV NS5A is a nonstructural protein that possesses no enzymatic activity, contains several CD8+ CTL and CD4+ T helper epitopes and yet reportedly is able to regulate viral replication and host cell interactions, providing a valid target for vaccine therapy. We have designed and produced a novel class of molecules (Chimigen® Vaccines) with attributes of viral antigen(s) and a xenotypic monoclonal antibody. These chimeric fusion proteins are designed to bind to specific receptors on DCs, 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 NS5A Vaccine, a fusion protein of NS5A antigen and a murine Fc fragment, has been cloned, expressed and purified. In this study, we have evaluated the ability of the vaccine to elicit antigen-specific T cell responses in PBMCs derived from patients with chronic HCV infection and in uninfected healthy donors.

ACKNOWLEDGEMENTS

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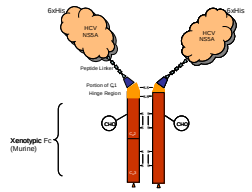


Unique Characteristics of the Chimigen® Vaccine

- Fusion protein comprised of antigen (the immune response domain – IRD) and the Fc portion of a xenotypic monoclonal antibody (the target binding domain - TBD)
- Adaptable platform; can incorporate any relevant antigen
- Unique chimeric design facilitates formation of an antibody-like structure
- 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 low doses
- The TBD facilitates binding of Chimigen® Vaccines to FcγRII (CD32) and macrophage mannose receptor (CD206) on DCs
- Antigen presentation via MHC class I and class II pathways

RESULTS

Chimigen® HCV NS5A Vaccine Has Been Expressed, Purified and Characterized

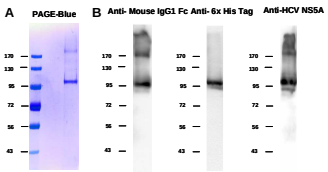


Figure 1. Purified Chimigen® HCV NS5A Vaccine. The fusion protein was purified using Ni-NTA and characterized by PAGE, Western blots using antibodies specific to different regions of the vaccine (A) 7.5% SDS PAGE PageBlue-stained, (B) Western blots using antibodies specific to 6xHis, IRD domain (anti-HCV antigens) and TBD (anti-Fc).

Chimigen® HCV NS5A Vaccine Binds to Immature DCs

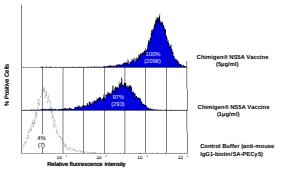
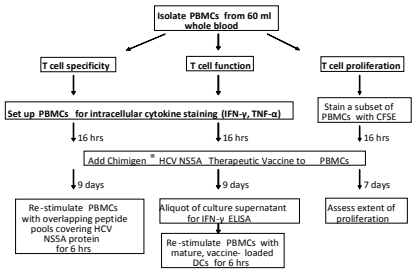


Figure 2. PBMC-derived immature DCs were incubated for 1 hr at 4°C with 1-50 µg/ml Chimigen® Vaccine. Bound protein was detected by flow cytometry following labeling with biotinylated anti-mouse IgG mAb and SA-PE-Cy5.

Assessment of T Cell Responses to the Chimigen® HCV NS5A Vaccine – Antigen Presentation Assay



Chimigen® HCV NS5A Vaccine Induces IFN-γand TNF-αProduction in $CD4^+$ and $CD8^+$ T Cells (PBMCs Derived From Healthy Donors)

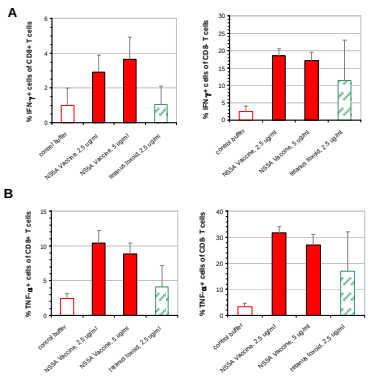


Figure 3. Th1 cytokine production by T cells (PBMCs derived from healthy donors). PBMCs were incubated with the vaccine for 11 days. PBMCs were then re-stimulated with mature, vaccine-loaded DCs for 6 hours. The percentage of T cells producing IFN-γ (A) or TNF-α (B) was determined by intracellular cytokine staining and flow cytometry.

Chimigen® HCV NS5A Vaccine Induces Proliferation of $CD4^+$ and $CD8^+$ T cells (PBMCs Derived From healthy Donors)

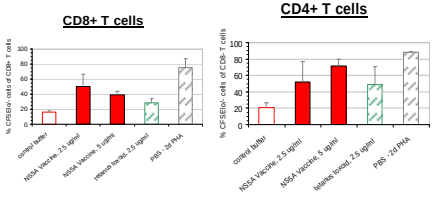


Figure 4. Proliferation of T cells (PBMCs derived from healthy donors). CFSE-labeled PBMCs were incubated with vaccine or vaccine components for 7 days. Cells were harvested and the percentage of $CFSE^{low/-}$ $CD8^+$ (A) and $CD4^+$ (B) T cells was determined by flow cytometry.

Chronic HCV Carrier Samples Used in the Study

Sample	Identifier	Age	Sex	HLA-A2 pos/neg
TRM	CHCV # 8	61	M	negative
WJL	CHCV # 12	58	M	negative
EAS	CHCV # 15	63	M	negative
GMW	CHCV # 18	55	M	negative
AWT	CHCV # 19	55	M	negative
LCH	CHCV # 21	52	F	negative
TSD	CHCV # 25	56	M	negative
DAN	CHCV # 7	53	M	positive
RMH	CHCV # 9	49	M	positive
BWZ	CHCV # 10	50/51	M	positive
RSM	CHCV # 11	49	M	positive
INM	CHCV # 13	58/59	F	positive
KJB	CHCV # 14	56	M	positive
BML	CHCV # 16	24	M	positive
BWW	CHCV # 17	52	M	positive
KRH	CHCV # 20	51/52	M	positive
JAP	CHCV # 22	58	F	positive
RCD	CHCV # 23	63	M	positive
WAQ	CHCV # 24	44	M	positive
BLN	CHCV # 26	40	F	positive

Chimigen® HCV NS5A Vaccine Induces proliferation of $CD8^+$ T cells and IFN-γSecretion by PBMCs Derived From Chronically Infected Donors

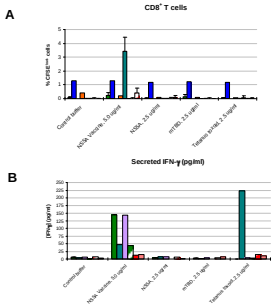


Figure 5. Proliferation and IFN-g secretion by PBMCs derived from HCV carriers were incubated with 1-10 ug/ml of vaccine for 7 days. (A) After 7 days, CSFE was added to the PBMCs and the cells were harvested later. (B) After 7 days, an aliquot of culture media was used for an IFN-γ ELISA. Each coloured bar represents a different donor.

Chimigenn® HCV NS5A Vaccine presentation by mature DCs results in increased intracellular IFN-γexpression in CD8+ and CD4+ T cells

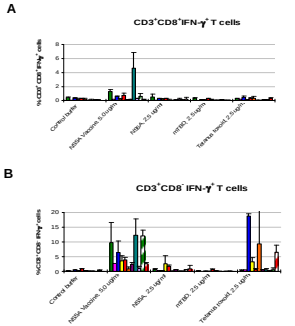


Figure 6. IFN-γ secretion by PBMCs derived from chronically infected donors. An aliquot of culture media was collected 9 days after the first stimulation with vaccine. The percentage of CD8+ (A) or CD4+ (B) T cells producing IFN-γ was determined by intracellular cytokine staining and flow cytometry. Each coloured bar represents a different donor.

Chimigen® HCV NS5A Vaccine Presentation by Mature DCs Results in Increased Intracellular TNF-αExpression in $CD8^+$ and $CD4^+$ T Cells

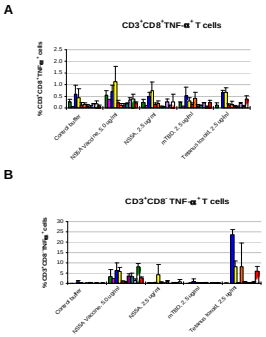


Figure 7. TNF-α production by T cells (PBMCs derived from chronically infected donors). PBMCs were incubated with the vaccine for 9 days. PBMCs were then re-stimulated with mature, vaccine-loaded DCs for 6 hours. The percentage of $CD8^+$ (A) or $CD4^+$ (B) T cells producing TNF-a was determined by intracellular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

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

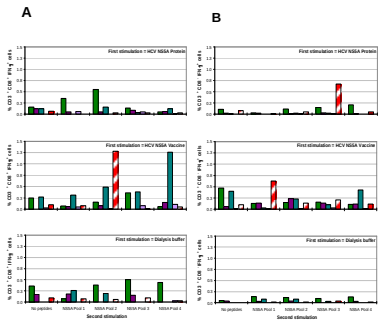


Figure 8. IFN-γ production by vaccine-treated T cells (PBMCs derived from chronically infected donors) which were re-stimulated with sets of overlapping peptides covering HCV NS5A protein. PBMCs were incubated with vaccine for 9 days. Pools of overlapping peptides were added to the PBMC cultures on day 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.

CONCLUSIONS

- The Chimigen® HCV NS5A vaccine has been cloned, expressed and purified
- The vaccine binds to immature DCs
- The vaccine induces proliferation of T cells from healthy donors and chronically infected donors
- Vaccine-treated PBMCs from healthy donors and chronically infected donors secrete IFN-γ
- The vaccine induces production of the Th1 cytokines IFN-γ and TNF-α by $CD8^+$ and $CD4^+$ T cells (healthy and chronically infected donors)
- Vaccine-stimulation results in HCV antigen-specific T cell expansion from PBMCs isolated from chronically infected donors
- The Th1 T cell immune response demonstrated in the *ex vivo* assays suggests that the Chimigen® HCV NS5A Vaccine may be a useful therapeutic vaccine for the treatment of chronic HCV infections