Novel Dendritic Cell – Receptor Targeted HBV and HCV Therapeutic Vaccines Induce Robust Immune Responses in HBV and HCV Chronic Carrier Peripheral Blood Mononuclear Cells Ex Vivo

Rajan George[1] , Allan Ma[1] , Dakun Wang[1], and Klaus Gutfreund[2]

[1]Paladin Biosciences, A Division of Paladin Labs Inc, 8223 Roper Road, Edmonton, Alberta, Canada, T6E 6S4, and [2]Department of Medicine, University of Alberta, Edmonton, Alberta, Canada.

e-mail: rajan.george@paladinbiosciences.com

ABSTRACT

BACKGROUND: Persistent exposure to viral antigens during chronic HBV and HCV infection may lead to functional impairment or deletion of virus-specific T cells. Therapeutic vaccines that induce strong host immune responses may help to resolve chronic HBV and HCV infections. A proprietary vaccine platform that uses the fusion of relevant antigen(s) with xenotypic antibody fragment Fc was developed. These chimeric molecules are designed to target the host immune system using specific receptors on dendritic cells (DCs) which are processed for presentation to T cells through both MHC class I and class II pathways to elicit antigen-specific cellular and humoral immune responses. The HBV therapeutic vaccine is a fusion protein of HBV antigens S1, S2 and Core and a murine Fc fragment, while the HCV therapeutic vaccine is a fusion protein of HCV NS5A and a murine Fc fragment. The vaccines were shown to elicit vaccine-specific T-cell and humoral immune responses in peripheral blood mononuclear cells (PBMCs) from uninfected healthy donors, *ex vivo*, and animals, *in vivo*. In this study, we have evaluated the ability of the vaccines to elicit antigen-specific T cell responses in PBMCs derived from patients with chronic HBV or HCV infection.

METHODS: The HBV and HCV therapeutic vaccines and their components were expressed in Sf9 insect cells and purified by Ni chelate affinity chromatography. PBMCs from chronically infected and uninfected donors were isolated and stimulated *ex vivo* with buffer, vaccine, antigen components or the xenotypic Fc fragment. Proliferation and production of Th1 cytokines IFN-γ and TNF-α by T-cells were measured by intracellular cytokine staining and flow cytometry. Preliminary studies to measure specificity of the immune response were done by re-stimulating PBMCs with pools of overlapping peptides corresponding to the antigen components. Production of IFN-γ and TNF-α in T-cells was measured by intracellular cytokine staining and flow cytometry.

RESULTS: A single stimulation with the vaccines yielded a higher percentage of proliferating T cells and cells secreting IFN-γ 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. When PBMCs were re-stimulated with pools of overlapping peptides corresponding to the S1, S2, Core or NS5A proteins, there was an increase in the HBV and HCV peptide pool-specific T cells producing IFN-γ and TNF-α. Vaccine stimulation resulted in HBV and HCV antigen-specific T cell expansion from PBMCs isolated from chronically infected donors.

CONCLUSIONS: The expansion of Th1 cytokine producing CD8+ and CD4+ T cells in PBMCs from chronically infected patients suggests that the HBV and HCV therapeutic vaccines may be good candidates for the treatment of chronic HBV and HCV infections.

INTRODUCTION

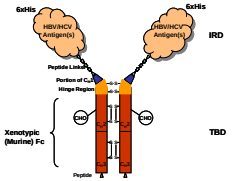
Hepatitis B virus (HBV) and Hepatitis C virus (HCV) have chronically infected hundreds of millions of people worldwide. Infected patients can remain asymptomatic for decades before developing liver cirrhosis and/or hepatocellular carcinoma. Unlike HBV, there is no vaccine available to prevent HCV infection. HCV patients currently receive a combination of interferon-α and ribavirin which is expensive, has substantial side effects and is effective only in a select group of patients. At present no approved therapeutic vaccine is available for the treatment of patients chronically infected with HBV or HCV. These are major unmet medical needs.

It is widely accepted that the host immune system plays a key role in the outcome of an HBV/HCV infection. The mechanism by which the HBV and HCV viruses escape attack by the host immune system is not fully understood. Most individuals that become exposed to HBV/HCV mount a strong and multi-specific CD4+ (helper) and CD8+ (cytotoxic) T cell response to the virus. These individuals develop only a self-limited infection. Adults exposed to HBV/HCV who mount a weak or undetectable and narrowly focused T cell response become chronic HBV/HCV carriers.

The failure of patients with HBV/HCV infection to initiate, maintain, and sustain a strong Th1 response may be due in part to the lack of proper presentation of the appropriate viral antigen to the host immune system. The success in eliminating the virus may 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.

Therapeutic vaccines which induce strong host immune responses may help to resolve chronic HBV/HCV infection. 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 antigen-specific, multi-epitopic cellular and humoral immune responses. Chimigen® HBV Multi-antigen (S1/S2/Core) Vaccine and Chimigen® HCV NS5A Vaccine, therapeutic vaccine candidates to treat chronic HBV and HCV infections, respectively, have been cloned, expressed and purified. In this study, we have evaluated the ability of the Chimigen® HBV Multi-antigen (S1/S2/Core) Vaccine and Chimigen® HCV NS5A Vaccine to elicit antigen-specific T cell responses in PBMCs derived from patients with chronic HBV or HCV infection and from uninfected healthy donors. In addition, the immune responses of these vaccine candidates in sheep and piglets, *in vivo*, are also presented.

Chimigen® Vaccine Recombinant Molecule



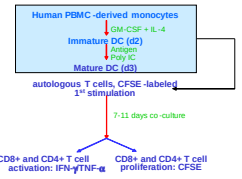
Unique Characteristics of the Chimigen® Vaccine

- 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 TBD and expression in insect cells which imparts non-mammalian glycosylation
- No added adjuvant
- Effective at small doses
- Chimigen® Vaccines bind to FcγRII (CD32) and macrophage mannose receptor (CD206) on DCs
- Antigen presentation via MHC class I and class II pathways
- Induces both cellular and humoral immune responses
- Useful in developing both prophylactic and therapeutic vaccines

METHODS

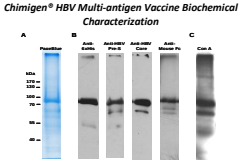
- Expression and Purification of Chimigen® Vaccines
  Proteins were cloned using the Bac-to-Bac system (Invitrogen) and expressed in Sf9 cells
  Expressed proteins were purified by Ni-NTA affinity chromatography and if necessary, followed by ion exchange chromatography
- Biochemical Characterization
  The purified fusion proteins were characterized by PAGE, glycostaining, and by Western blots using antibodies specific to different regions of the vaccine
- Immunology, *ex vivo*
  Evaluation *ex vivo* using T cells and autologous dendritic cells from normal or chronically HBV /HCV infected donor PBMCs
  T cell activation (intracellular IFN-γ)
  Antigen-specific CD8+ T cells (pentamer, peptide pools)
- Immunology, *in vivo*, in sheep (HBV vaccine) and piglets (HCV vaccine)
  Chimigen® *HBV Multi-antigen Vaccine study in sheep*
  8 lambs per group, 6-8 weeks old
  Immunization (s.c.) three times at four week intervals
  Chimigen® Vaccine (5, 20 or 50 μg per sheep), or carrier buffer
  Chimigen® *HCV NS5A Vaccine study in piglets*
  5 piglets per group, 4 weeks old
  Immunization (s.c.) three times, at two week intervals with
  Chimigen® Vaccine (5, 10, 50 μg per piglet), or carrier buffer

Antigen Presentation Assay, ex vivo



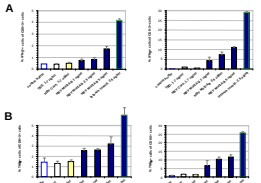
RESULTS

Chimigen® HBV Multi-antigen Vaccine Biochemical Characterization



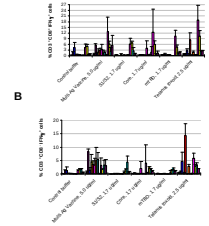
Chimigen® HBV Multi-antigen Vaccine was purified using Ni-NTA chromatography and characterized by PAGE, glycostaining, and by Western blots using antibodies specific to different regions of the vaccine. (A) 7.5% SDS PAGE PageBlue-stained, (B) Western blots using antibodies specific to 6xHis, IRD domain (anti-HBV Ags) and TBD (anti-Fc) and (C) ConA-HRP stain.

Chimigen® HBV Vaccine Induces IFN-γand TNF-α Production in CD4+ and CD8+ T Cells (PBMCs Derived from Healthy Donors)



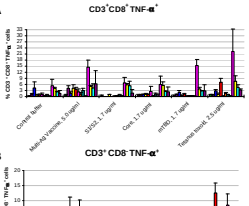
Th1 cytokine production by T cells (PBMCs derived from healthy donors). PBMCs were incubated with the vaccine for 10 days. PBMCs were then re-stimulated with vaccine-loaded DCs for 6 hours. The percentage of T cells producing IFN-γ (A) or TNF-α (B) was determined by intracellular cytokine staining followed by flow cytometry.

Chimigen® HBV Vaccine Presentation by Mature DCs Results in Increased Intracellular IFN-γExpression in CD8+ and CD4+ T Cells (PBMCs Derived from Chronic HBV Carriers)



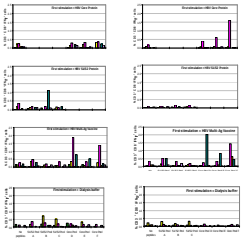
Intracellular IFN-γ production by CD3+CD8+ T cells (A) and CD3+CD8- (CD4+) T cells (B) isolated from chronic HBV carriers following re-stimulation with the Chimigen® HBV Vaccine. Each data point represents the average of triplicate assays

Chimigen® HBV Vaccine Presentation by DCs Results in Increased Intracellular TNF-αExpression in CD8+ and CD4+ T Cells from Chronically HBV-infected donors



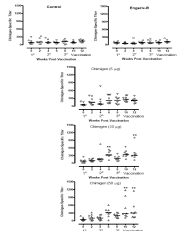
TNF-α production by T cells (PBMCs derived from chronically HBV infected donors). PBMCs were incubated with the vaccine for 9 days and then re-stimulated with vaccine-loaded DCs for 6 hours. The percentage of CD8+ (A) or CD4+ (B) T cells producing TNF-α was determined by intracellular cytokine staining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

Chimigen® HBV Multi-antigen Vaccine Induces Antigen-Specific CD8+ and CD4+ T Cell Intracellular IFN-γExpression



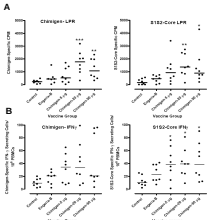
IFN-γ production by vaccine-treated T cells (PBMCs derived from chronically HBV infected donors) that were re-stimulated with sets of overlapping peptides covering HBV S1, S2 or Core proteins. PBMCs were incubated with vaccine for 9 days. Pools of overlapping peptides were added to the PBMC cultures 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.

Chimigen® HBV Multi-antigen Vaccine Induced Vaccine-specific Antibody Responses in sheep Following Third Immunization



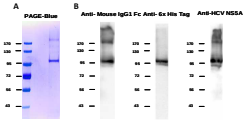
Lambs (8/group) were injected subcutaneously with carrier buffer, Engerix-B (20μg), Chimigen® HBV Vaccine (5μg,20μg and 50μg/animal/injection). Serum antibody titres were determined with an indirect ELISA with wells coated with Chimigen® HBV Vaccine. Titres are expressed as the reciprocal of the serum dilution which gave an optical density greater than the negative cut-off value. Data presented are values for individual animals within each group and the horizontal bar represents the median value for each group. *P<0.05, ** P<0.01

Chimigen® HBV Multi-antigen Vaccine Induces Antigen-Specific T Cell Proliferation and IFN-γ Secretion by PBMCs Derived from Immunized Sheep



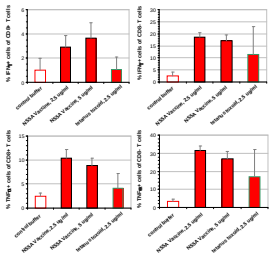
Proliferation of T cells (A) in PBMCs derived from immunized sheep (8/group). PBMCs from immunized sheep were re-stimulated *ex vivo* with vaccine or its components for 7 days. After 7 days, [^{3}H]thymidine was added to the PBMCs and the cells were harvested 18 hrs later. Incorporation of [^{3}H]thymidine was measured using a scintillation counter. IFN-γ secretion (B) by PBMC-derived T cells from immunized sheep. PBMCs were incubated with vaccine or its components for 9 days *ex vivo*. IFN-γ secreting cells in culture media was determined by IFN-γ ELISPOT assay. Each dot represents an individual sheep.

Chimigen® HCV NS5A Vaccine has Been Purified and Characterized



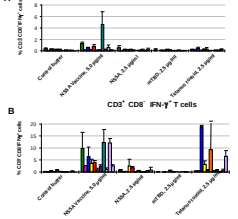
Chimigen® HCV NS5A Vaccine was purified using Ni-NTA chromatography and characterized by PAGE, Western blots using antibodies specific to different regions of the vaccine and glycostaining. (A) 7.5% SDS PAGE PageBlue-stained, (B) Western blots using antibodies specific to TBD (anti-Fc), 6xHis and IRD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

Chimigen® HCV NS5A Vaccine Induces IFN-γand TNF-α Production in CD4+ and CD8+ T Cells (PBMCs Derived from Uninfected Donors)



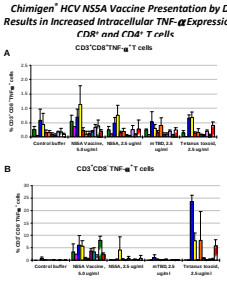
Th1 cytokine production by T cells (PBMCs derived from uninfected donors). PBMCs were incubated with the vaccine for 11 days. PBMCs were then re-stimulated with mature, vaccine-loaded DCs for 6 hours. The percentage of T cells producing IFN-γ or TNF-α was determined by intracellular cytokine staining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

Chimigen® HCV NS5A Vaccine Presentation by DCs Results in Increased Intracellular IFN-γExpression in CD8+ and CD4+ T Cells (PBMCs Derived from Chronically HCV-Infected Donors)



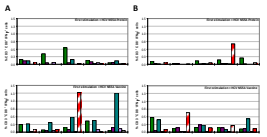
IFN-γ production by T cells (PBMCs derived from chronically HCV infected donors). PBMCs were incubated with the vaccine for 9 days and then re-stimulated with vaccine-loaded DCs for 6 hours. The percentage of CD8+ (A) or CD4+ (B) T cells producing IFN-γ was determined by intracellular cytokine staining and flow cytometry.

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



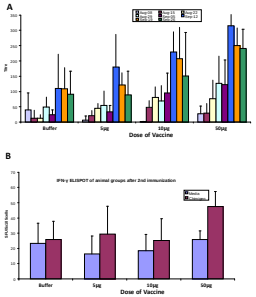
TNF-α production by T cells (PBMCs derived from chronically HCV infected donors). PBMCs were incubated with the vaccine for 9 days and then re-stimulated with vaccine-loaded DCs for 6 hours. The percentage of CD8+ (A) or CD4+ (B) T cells producing TNF-α was determined by intracellular cytokine staining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

Chimigen® HCV NS5A Vaccine Induces HCV Antigen-specific CD8+ and CD4+ T Cell Intracellular IFN-γ Expression



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 proteins. 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.

Chimigen® HCV NS5A Vaccine Induces Antibody and Cellular Immune Responses In Piglets



A. Antibody titration. Piglets were injected subcutaneously with either carrier buffer or Chimigen® HCV Vaccine (5μg, 10μg and 50μg/animal/injection). Serum antibody titres were determined using an indirect ELISA after coating the wells with Chimigen® HCV Vaccine. Titres are expressed as the reciprocal of the serum dilution which gave an optical density greater than the negative cut-off value.

B. IFN-γ-secretion by PBMCs derived from immunized piglets. PBMCs were incubated with the vaccine for 9 days *ex vivo*. IFN-γ ELISPOT assay was used to determine IFN-γ secretion by the cells.

CONCLUSIONS

- The Chimigen® HBV Multi-antigen Vaccine and Chimigen® HCV NS5A Vaccine were produced in Sf9 insect cells and purified
- Chimigen® HBV and HCV Vaccines bound to immature DCs
- Stimulation by both vaccines resulted in antigen-specific expansion of PBMC-derived T cells from both chronically HBV and HCV infected donors, *ex vivo*
- Both vaccines induced the production of the Th1 cytokines IFN-γ and TNF-α by CD8+ and CD4+ T cells from uninfected and infected donors
- Both vaccines induced cellular and humoral immune responses in animals
- The results from antigen presentation assays *ex vivo* using human PBMCs and the animal studies *in vivo* suggest that the Chimigen® HBV Multi-antigen Vaccine and Chimigen® HCV NS5A Vaccine have excellent potential as therapeutic vaccines for the treatment of chronic HBV and HCV infections

ACKNOWLEDGEMENTS

Financial support from NRC-IRAP Canada, AHFMR and AIF is gratefully acknowledged. We thank Dr. Brian Ma for many helpful discussions and Drs. Qiang Liu & Philip Griebel, VIDO for their help with the sheep and piglet studies.

HepDART 2009 Frontiers in Drug Development for Viral Hepatitis, December 6-10, 2009 The Fairmont Orchid Kohala Coast, Hawaii, USA