Novel Dendritic Cell Receptor Targeted HBV Therapeutic Vaccine Induces Robust Immune Responses in HBV Chronic Carrier Peripheral Blood Mononuclear Cells

Rene E. Déry, , Allan Ma[1] , Dakun Wang[1], Yun Xia[1] Klaus Gutfreund[2] and Rajan George[1]

[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; Exposure to viral antigens during chronic HBV infection may lead to functional impairment or deletion of virus-specific T cells. Therapeutic vaccines that induce strong host immune responses may therefore resolve chronic HBV infection. We developed a therapeutic HBV vaccine which is a fusion of HBV S1, S2, Core and murine Fc fragment. This vaccine targets the host immune system using specific receptors on dendritic cells (DCs). We have previously shown that the vaccine elicits *ex vivo* antigen-specific cytotoxic and humoral immune responses in healthy human peripheral blood mononuclear cells (PBMCs), and in animals *in vivo*. The current study evaluates the vaccine's ability to elicit antigen-specific T cell responses in PBMCs derived from patients with chronic HBV infection.

METHODS: The vaccine was expressed in Sf9 cells and purified by affinity chromatography. PBMCs from either uninfected or HBV chronically infected donors were stimulated *ex vivo* by co-culturing with vaccine-loaded mature DC. The resulting T-cell proliferation and production of IFN-γ, TNF-α, and Granzyme B was measured by intracellular cytokine staining. Antigen-specificity of vaccine-induced immune response was measured by assessing IFN-γ and TNF-α production of vaccine primed PBMCs following re-stimulation with pools of HBV S1/S2/Core overlapping peptides. The HBV-specific cytotoxic lymphocyte (CTL) response was measured in HBV chronically infected patient PBMCs *ex vivo* using the real-time cell microelectronic sensor (RT-CES) system. Vaccine-loaded DCs were used as antigen-presenting cells when co-cultured with naïve T cells, or as target cells when co-cultured with vaccine-primed T cells.

RESULTS: Vaccine stimulation induced proliferation and IFN-γ-secretion in uninfected donor naïve T cells. Vaccine re-stimulation of primed T-cells induced increased production of IFN-γ and TNF-α by CD8+ and CD4+ T cells. Vaccine stimulation of PBMC from HBV chronic carriers resulted in HBV antigen-specific T cell expansion and cytokine production. When vaccine primed PBMCs from HBV carriers were re-stimulated with pools of overlapping peptides, there was a an antigen-specific increase in T cell IFN-γ and TNF-α secretion. Interestingly, vaccine stimulation of PBMCs from HBV infected patients stimulated Granzyme B production by CD4+CD25- cells (Tresp), accompanied by an increase in Annexin V+ staining on CD4+CD25+ cells (Tregs). In our dynamic CTL killing assay, co-culture of vaccine-primed HBV chronic carrier T cells with mature DCs resulted in a dose-, time- and T cell-dependent decrease in cell DC viability.

CONCLUSION: The vaccine-dependent expansion of IFN-γ, TNF-α producing CD8+ and CD4+ T cells as well as CTL killing of DC and Tresp-induced apoptosis of Tregs in HBV infected donor PBMCs suggests that the vaccine can break HBV tolerance in chronic HBV carriers by inducing strong immune responses which include functional activation of Tresp-dependent apoptosis of Tregs. Thus, our HBV therapeutic vaccine may be a good candidate for the treatment of chronic HBV infections.

INTRODUCTION

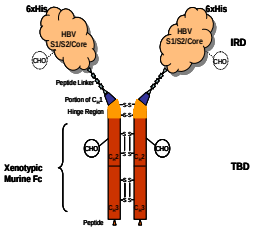
More than 370 million people are chronically infected with the Hepatitis B virus (HBV). Chronic infection often remains asymptomatic for decades before patients develop liver cirrhosis and/or hepatocellular carcinoma. Only a minority of patients achieve durable treatment responses with the currently approved antiviral therapies. At present, there is no therapeutic vaccine available for patients chronically infected with HBV. Novel therapies for hepatitis B remain a major unmet medical need.

The host immune system plays a key role in the outcome of an HBV infection. The mechanism by which the HBV virus escapes attack by the host immune system is not fully understood. Most individuals that become exposed to HBV 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 who mount a weak or undetectable and narrowly focused T cell response become chronic HBV carriers.

The failure of patients with HBV 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 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 Vaccine comprised of the S1/S2/Core HBV antigens fused to the Fc portion of a murine IgG xenotypic antibody has been cloned, expressed and purified. We have shown in previous studies that this vaccine binds to DCs and activates strong humoral and cell-mediated immune responses in peripheral blood mononuclear cell (PBMC)-derived T cells *ex vivo*. We have also shown that the Chimigen® HBV Multi-antigen Vaccine generates strong humoral and cell-mediated immune responses in an animal model *in vivo*. In this study, we have evaluated the ability of the Chimigen® HBV Multi-antigen Vaccine to elicit antigen-specific T cell responses in PBMCs derived from patients with chronic HBV infection.

Chimigen® Vaccine Recombinant Molecule



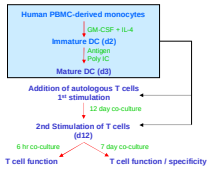
Unique Characteristics of the Chimigen® Vaccine

- 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 low doses
- Chimigen® Vaccines bind to FcγRII (CD32) and macrophage mannose receptors (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

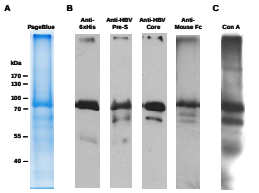
- Expression and Purification of Chimigen® Vaccines
  Proteins were cloned using the Bac-to-Bac system (Invitrogen) and expressed in Sf9 cells
- Expressed proteins were purified by Nickel affinity chromatography
- Biochemical Characterization
  The purified fusion proteins were characterized by PAGE, 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 uninfected or chronically HBV infected donor PBMCs
  - T cell activation (Proliferation)
  - T cell effector function (IFN-γ, TNF-α production)
  - CTL induction (Perforin, Granzyme B production)
  - HBV antigen-specific CD8+ T cells (overlapping peptide pools)
- Real-time cell microelectronic sensor (RT-CES) CTL killing assay

Antigen Presentation Assay, *ex vivo*



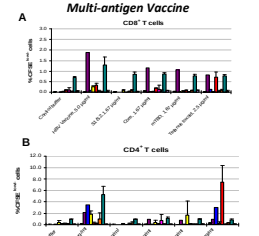
RESULTS

Chimigen® HBV Multi-antigen Vaccine Biochemical Characterization



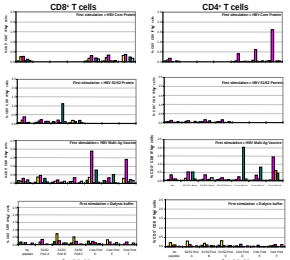
Chimigen® HBV Multi-antigen Vaccine was purified using Nickel affinity chromatography and characterized by PAGE, glycostaining, and by Western blots using antibodies specific to different regions of the vaccine. (A) 7.5% SDS PAGE PageBlue-stained, (B) Western blots using antibodies specific to 6xHis,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

T Cell Proliferation Induced by Chimigen® HBV Multi-antigen Vaccine



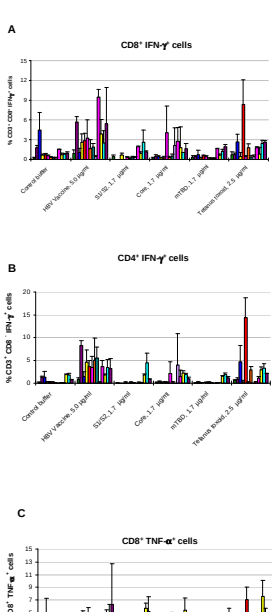
Induction of T cell proliferation in (A) CD8+ and (B) CD4+ by the Chimigen® HBV Multi-antigen Vaccine in PBMCs isolated from nine chronically-infected donors. Each color bar represents one individual donor.

T cell Priming With Vaccine-loaded mDC Results in Antigen-specific T Cells IFN-γ Production in Response to HBV S1/S2 or Core Overlapping Peptide Restimulation



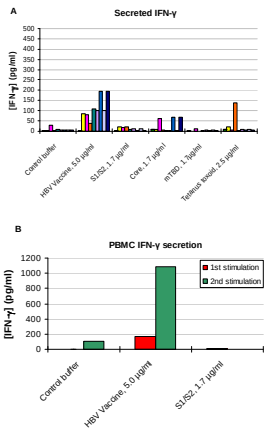
Intracellular IFN-γ production by CD3+CD8- (CD4+) and CD3+CD8+ (CD8+) T cells isolated from chronic HBV carriers following re-stimulation with the HBV S1/S2 or core peptides. Each color bar represents one individual donor.

Induction of IFN-γ and TNF-α Expression by the Chimigen® HBV Multi-antigen Vaccine in PBMCs From Chronic HBV Carriers



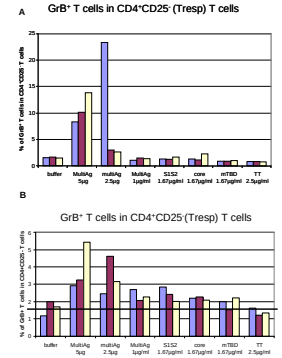
Intracellular IFN-γ and TNF-α production by CD3+CD8+ (CD8+) (Panel A and C) and CD3+CD8- (CD4+) (Panel B and D) T cells isolated from chronically infected HBV patients following re-stimulation with the Chimigen® HBV Multi-antigen Vaccine. Each color bar represents one individual donor. Data is presented as the average of triplicate wells.

Induction of IFN-γ Secretion by the Chimigen® HBV Multi-antigen Vaccine in PBMCs From Chronic HBV Carriers



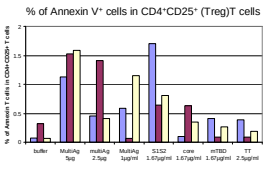
IFN-γ secretion induced by the Chimigen® HBV Multi-antigen Vaccine in PBMCs isolated from chronic HBV carriers following (A) one stimulation or (B) two stimulations with the vaccine. Data shown is derived from a single experiment.

Induction of Granzyme B by the Chimigen® HBV Multi-antigen Vaccine in PBMCs From Chronic HBV Carriers



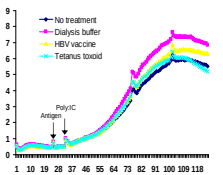
Granzyme B production induced by the Chimigen® HBV Multi-antigen Vaccine in (A) PBMCs isolated from chronically HBV infected patient #14 and (B) PBMCs isolated from chronically HBV infected patient #23. Data presented as individual values from triplicate wells.

Induction of Treg Apoptosis by the Chimigen® HBV Multi-antigen Vaccine in PBMCs from Chronic HBV Carriers



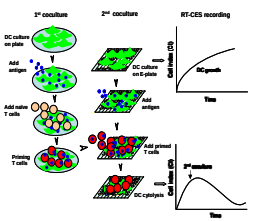
Apoptotic cell death of Treg cells in PBMCs isolated from chronic HBV carrier # 23 after stimulation with Chimigen® HBV Multi-antigen Vaccine. Data is presented as individual values of triplicate wells.

Induction of DC Death by Chimigen® HBV Multi-antigen Vaccine Does Not Occur in the Absence of T Cells.



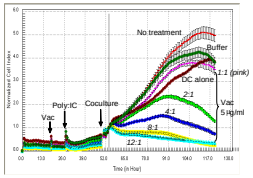
Addition of the vaccine (5 µg/ml) to the immature DC culture followed by Poly I:C in the absence of activated T cells did not induce DC cell death.

Real-time Cell Electronic Sensor (RT-CES) Cytotoxic T Lymphocyte (CTL) Assay



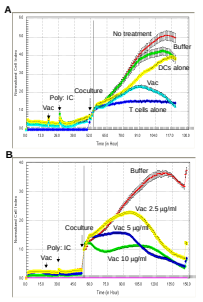
Schematic representation of the antigen-specific CTL response induced by two subsequent co-cultures and the assessment of the response measured using the RT-CES system.

DC Death is T Cell-dependent



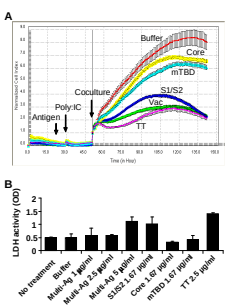
T cell density-dependent CTL response. Immature DCs (1 × 10^5) were seeded in wells of E-plates. 20 h after plating, immature DCs were treated with vaccine (5 µg/ml) or dialysis buffer followed by poly I:C to induce maturation. At the indicated time point, vaccine-activated T cells at 1 to 12 × 10^5/well were added to antigen-loaded mature DC cultures, respectively. Effector-to-target (E:T) ratios are indicated in the figure. Changes in Cell Index were monitored every 1 h by the RT-CES system. The decrease in DC cell index correlated with an increase in T cell densities.

Chimigen® Multi-antigen Vaccine Induces CTL-mediated DC Cytotoxicity in Uninfected Donor PBMCs



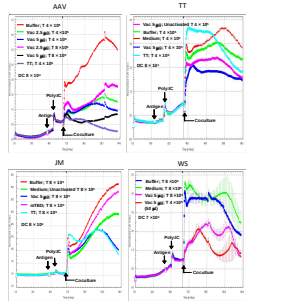
Dynamic monitoring of vaccine-induced T cell-mediated CTL response using the RT-CES system in uninfected donors. Immature DCs (1 × 10^5/well) were seeded and cultured in E plates. After 20 h, immature DCs were treated with the vaccine at (A) 5 µg/ml or (B) 2.5, 5 or 10 µg/ml followed by maturation with poly I:C. At the indicated time point, vaccine-activated purified T cells at 4 × 10^5 cells/well were added to the wells containing DCs. Changes in Cell Index were monitored every 1 h by the RT-CES system. A vaccine dose-dependent drop in Cell Index correlating with death and detachment of DC can be observed.

Vaccine-activated T cells Induce Antigen-specific Cell Killing



Antigen-specific killing of Vaccine- or vaccine component-loaded target cells. Co-culture of vaccine-activated T cells with the corresponding antigen-loaded mature DCs resulted in antigen-specific cytotoxic responses measured by (A) RT-CES system or (B) Lactate dehydrogenase (LDH) release.

Chimigen® Multi-antigen Vaccine-Induced CTL-mediated Cytotoxicity in HBV Chronic Carriers



Dynamic monitoring of vaccine-induced T cell-mediated cytotoxicity in 6 HBV chronic carriers (AAV, TT, JM, WS, SHL, RSL) using the RT-CES system. Immature DCs (0.7 to 1 × 10^4/well) were seeded and cultured on E-plates. 20 h after plating, immature DCs from infected donors were treated with vaccine, vaccine components or TT followed by Poly I:C. At the indicated time point, vaccine-primed purified T cells (AAV and SHL) or whole PBMCs (TT, JM, WS and RSL) at 4 × 10^5 or 8× 10^5 cells/well (unless otherwise indicated) were added into the mature DC cultures. Changes in Cell Index were monitored at 1 h intervals using the RT-CES system. A marked decrease in Cell Index in vaccine-treated group compared to relative controls occurred in four patients (AAV, TT, JM, WS). In one patient (SHL) no decrease in Cell Index occurred, and in RSL a decrease in Cell Index occurred accompanied by T cell death.

SUMMARY

- A novel HBV DC-targeting Therapeutic Vaccine (Chimigen® HBV Vaccine) was produced in Sf9 insect cells and purified
- PBMC derived DC/T cells from both uninfected and chronically HBV infected donors were used to evaluate immune responses to the vaccine
- Vaccine-loaded mature DCs stimulate expansion of PBMC-derived T cells *ex vivo* from both uninfected donors and patients chronically infected with HBV
- T cells from chronically HBV-infected patients secrete IFN-γ following culture with vaccine-loaded mature DCs
- IFN-γ secretion in T cells activated by vaccine-loaded mature DC increases following a second stimulation with vaccine-loaded mDCs
- Vaccine-primed T cells express IFN-γ and TNF-α in response to second stimulation with HBV S1/S2/Core overlapping peptides
- The vaccine induces IFN-γ and TNF-α expression, and Granzyme B release by CD8+ and CD4+ T cells of patients chronically infected with HBV
- The vaccine activates CD4+CD25- (T responder) cells which induce apoptosis of CD4+CD25+ (T regulatory) cells
- Vaccine-activated T cells exhibit cytotoxic T lymphocyte activity towards DCs derived from both uninfected and chronically HBV-infected donors

CONCLUSION

Ex vivo testing using human PBMCs from uninfected or chronically HBV-infected donors suggest that the Chimigen® HBV Multi-antigen Vaccine has excellent potential as a therapeutic vaccine for the treatment of chronic HBV infections.

ACKNOWLEDGEMENTS

Financial support from NRC-IRAP Canada, NSERC and Alberta Innovates Technology Futures is gratefully acknowledged. René Déry is a recipient of the Alberta Innovates Technology Futures R&D Associate Award.