Chimigen® HBV Therapeutic Vaccine Induces a Robust Immune Responses in Sheep

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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® HBV Therapeutic Vaccine, a fusion protein of HBV S1 and S2 surface antigen fragments, core and a murine Fc fragment, has been produced in insect cells, purified and characterized. In antigen presentation assays *ex vivo*, using human naïve as well as HBV carrier peripheral blood mononuclear cells (PBMCs), the vaccine induced the expansion of HBV antigen-specific CD8+ and CD4+ T cells producing Th1 cytokines and the expanded CD8+ T cells expressed perforin and granzyme B.

In the present study, we assessed the immune responses to the Chimigen® HBV Therapeutic Vaccine or its components in sheep. Sheep were immunized three times with either carrier buffer or Chimigen® Vaccine (5, 20 or 50µg per animal) at four week intervals. Engerix-B (GSK) was used as a positive control for humoral response. Following vaccinations, PBMCs were isolated from sheep blood and the cellular immune responses were determined *ex vivo* by stimulating them with buffer, vaccine, or the vaccine components (HBV antigens S1/S2/Core or the xenotypic Fc fragment). Analysis of cell-mediated immune responses in PBMCs confirmed that the vaccine induced dose-dependent S1/S2/Core-specific proliferative responses and the secretion of IFN-γ. Analysis of serum antibody responses by ELISA showed that the vaccine induced dose-dependent antibody responses to the S1/S2/Core protein following the second and third immunizations, with kinetics similar to the HBsAg-specific antibody response induced by Engerix-B, in the absence of adjuvant. The cellular responses in PBMCs appeared to be much stronger than that of cells from the lymph node draining the vaccination site. These results suggest that the Chimigen® HBV Therapeutic Vaccine may be a good candidate for the treatment of chronic HBV infections.

INTRODUCTION

Chronic hepatitis B virus (HBV) infection is a major healthcare issue. It is estimated that 350 million people worldwide are chronically infected with HBV. Antivirals can reduce the replication of the virus, but help from the host immune system is necessary for the elimination of the infection. Prophylactic vaccines based on HBV surface antigen have been very effective in providing immunity against HBV infection, however, these vaccines are ineffective against chronic infection. Therapeutic vaccines for the treatment of chronic HBV infections are not available.

HBV clearance is associated with a strong humoral (anti-envelope protein antibodies) and cellular immune response. Antibodies neutralize circulating virus particles and CD8+ T cells kill infected hepatocytes through cytolytic and non-cytolytic (cytokine production) mechanisms. CD4+ T cells are integral in the activation of effector cytotoxic T cells and antibody-producing B cells and are also robust producers of cytokines. Therefore, strong HBV-specific CD8+ and CD4+ T cell responses that result in a Th1 cytokine production profile correlate with resolution of infection.

Persistent exposure to viral antigens during chronic hepatitis B infection may lead to functional impairment or deletion of virus-specific T cells. The exact mechanism for impaired immune function in chronic HBV infections is unknown. Many mechanisms may be involved in altering the repertoire of HBV-specific immunity. It is likely that functional reconstitution of immunity will be very complex and may involve inhibiting viral replication and activation of both arms of the immune response.

Therapeutic vaccines that induce a strong host immune response 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 resulting in the generation of both cellular and humoral immune responses. The Chimigen® HBV Multi-Antigen Vaccine, a fusion protein of HBV antigens 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 antibody and T cell responses in sheep. Antigen-specific T cell responses produced by the vaccine in human PBMCs *ex vivo*, are also presented.

ACKNOWLEDGEMENTS

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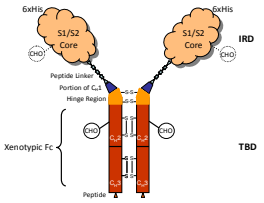


Unique Characteristics of the Chimigen® Vaccine

- 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
- 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® HBV Multi-Ag Vaccine was Expressed, Purified and Characterized

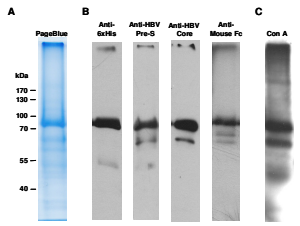


Figure 1. Purified Chimigen® Vaccine. The fusion protein was purified using Ni-NTA and characterized by PAGE, glycostaining, and by Western blots using antibodies specific to different regions of the vaccine. (A) 7.5% SDS PAGE PageBlue-stained, (B) Western blots using antibodies specific to 6xHis, IRD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

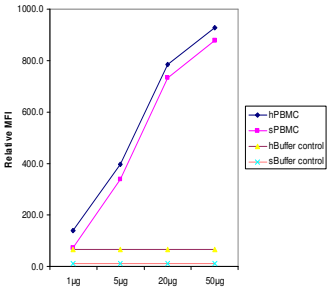
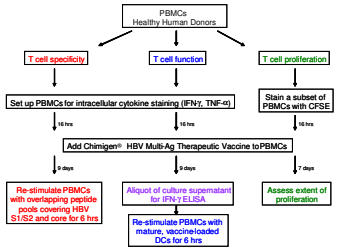


Figure 2. Cell-based binding assay. Human PBMC-derived immature DCs and sheep PBMCs were incubated for 1 hr at 4°C with 1-50 µg/ml Chimigen® Vaccine. Bound protein was detected by flow cytometry following labeling with biotinylated anti-mouse IgG mAb and SA-PE-Cy5.

Assessment of the T Cell Responses to the Chimigen® HBV Multi-Ag Vaccine Antigen Presentation Assay



Chimigen® HBV Multi-Ag Vaccine Induces Proliferation and IFN-γ Secretion by PBMCs Derived From Healthy Donors

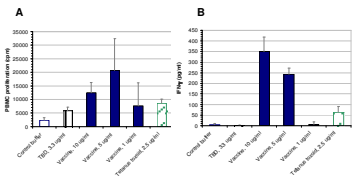


Figure 3. Proliferation and IFN-γ secretion by PBMCs derived from healthy donors. PBMCs were incubated with 1-10 µg/ml of vaccine for 7 days. (A) After 7 days, [3H]thymidine was added to the PBMCs and the cells were harvested 18 hrs later. Incorporation of [3H]thymidine was measured using a scintillation counter. (B) After 7 days, an aliquot of culture media was used for an IFN-γ ELISA.

Chimigen® HBV Multi-Ag Vaccine Induces IFN-γ and TNF-α Production in CD4+ and CD8+ T Cells (PBMCs Derived From Healthy Donors)

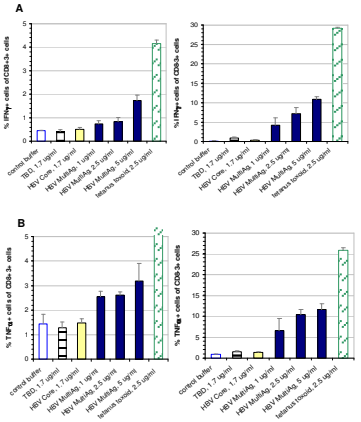


Figure 4. Th1 cytokine production by T cells (PBMCs derived from healthy donors). PBMCs were incubated with the vaccine for 10 days. PBMCs were then re-stimulated with mature, vaccine-loaded DCs for 6 hours. The percentage of T cells producing IFN-γ (A) or TNF-α (B) was determined by intracellular cytokine staining followed by flow cytometry.

Schedule of Immunization of Sheep with Chimigen® HBV Vaccine and Sample Collection

Exper. week	Vaccination	Serum Collection	PBMC/LN Collection	Comments
Day -1				Identify and assign animals to exp. Groups
Week 0	√	√		Collect samples prior to vaccination
Week 2		√		
Week 4	√	√		
Week 6		√		
Week 7				
Week 8	√	√	√ (PBMC)	
Week 10		√		
Week 12		√	√ (PBMC-Day 83) √ (LN-Day 85)	Terminate experiment

Chimigen® HBV Multi-Ag Vaccine Induces Proliferation of PBMCs from Immunized Sheep

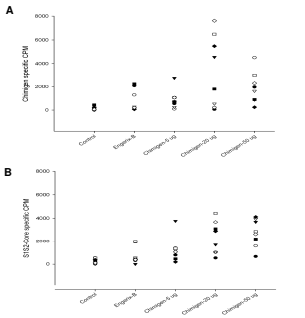


Figure 5. Proliferation of T cells from PBMCs derived from immunized sheep. PBMCs from immunized sheep were re-stimulated *ex vivo* with vaccine (A) or one of its components (B) for 7 days. After 7 days, [3H]thymidine was added to the PBMCs and the cells were harvested 18 hrs later. Incorporation of [3H]thymidine was measured using a scintillation counter.

Chimigen® HBV Multi-Ag Vaccine Induces IFN-γ Secretion by PBMCs from Immunized Sheep

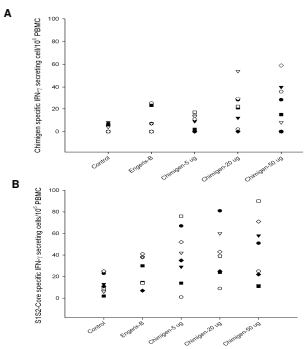


Figure 6. IFN-γ secretion by PBMCs derived from immunized sheep. PBMCs were incubated with vaccine (A) or one of its components (B) for 9 days *ex vivo*. An aliquot of culture media was collected on day 9. IFN-γ secretion in culture media was determined by IFN-γ ELISPOT assay. Each dot represents an individual sheep.

Chimigen® HBV Multi-Ag Vaccine Induced Vaccine-specific and S1/S2/Core-Specific Antibody Responses in PBMCs after Final Immunization.

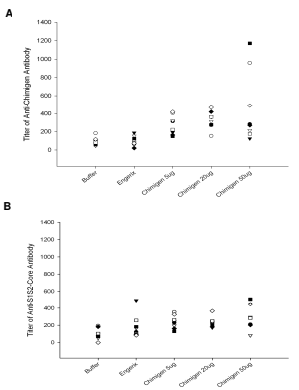


Figure 7. Determination of Antibody titer and specificity by ELISA. Serum from immunized sheep was incubated with the vaccine (A) or S1/S2/Core antigen (B) followed by HRP-conjugated secondary antibody.

CONCLUSIONS

- The Chimigen® HBV Multi-Ag vaccine has been successfully cloned, expressed and purified
- The vaccine binds to both human and sheep PBMCs
- The vaccine induces antigen-specific antibody production in sheep
- The vaccine induces proliferation of both human and sheep T cells
- Vaccine-treated PBMCs from both humans and sheep secrete IFN-γ
- The vaccine induces production of the Th1 cytokines IFN-γ and TNF-α by CD8+ and CD4+ T cells from healthy human donors
- PBMCs from immunized sheep showed expansion of HBV antigen-specific T cells , following re-stimulation with the vaccine *in vitro*
- The Th1 T cell immune response demonstrated in this sheep study suggests that the Chimigen® HBV Multi-Ag Vaccine may be a useful therapeutic vaccine for the treatment of chronic HBV infections

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