RECOMBINANT YEAST THERAPEUTIC VACCINES EXPRESSING HEPATITIS B VIRUS (HBV) X, S, AND CORE ANTIGENS GENERATE ANTI-SPECIFIC T CELL RESPONSES AND TUMOR PROTECTION IN MICE

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Introduction

Hepatitis B virus (HBV) is the leading cause of chronic infection of the liver in the world. World Health Organization (WHO) has estimated that 350 million people are chronic carriers of HBV, of whom more than 600,000 die from liver-related diseases each year in the United States, approximately 800,000 to 1.4 million Americans are chronic hepatitis B virus carriers, resulting in approximately 3,000 deaths annually.

Chronic HBV is characterized by adaption of T cell responses against viral antigens. Direct etiologic analysis has suggested that HBV hepatitis may be associated with chronic inflammatory and immune response. Tarmogens and T cells have demonstrated a good functional profile in animal models and humans, but in vitro to in vivo translation is highly challenging. To successfully translate in vitro to in vivo, Tarmogens are designed in a way that they could be used in an attractive antigen format.

Eighteen HBV Tarmogens expressing variations of HBV X, S, Pol and Core antigens were engineered to express high quantities of disease-related proteins inside the yeast cell. These Tarmogens have been shown to generate robust, antigen-specific CD8+ and CD4+ T cell responses in cancer and chronic infectious diseases. Tarmogens have demonstrated a good translation profile in various preclinical studies and in over 300 trials in multiple oncology and hepatitis clinical studies.

Eight HBV Tarmogens expressing variations of HBV X, S, Pol and Core antigens were captured and evaluated. Eight additional constructs were created using SCore as a base, aiming to expand the antigen repertoire of the product by attaching Pol and X variants. When these new constructs were expressed in yeast, a greater response for X-SCore than Yvec-immunized mice.

Evaluation of the first ten Tarmogens revealed favorable protein expression, immunogenicity and growth rate. One Tarmogen contains a chimera of HBV X, S and Core (GI-13009 or “SCore”), and a second contains a chimera of HBV X, S and Core antigens (GI-13022).

Murine in vitro immunogenicity – BALB/c mice

To evaluate the HBV ELISPOT response found in BALB/c mice extends to other mouse genetic backgrounds, C57BL/6 mice were immunized, T cells retrieved and assayed by ELISPOT as described previously. X-SCore vaccination elicited robust IFN-γ ELISPOT responses in lymph node cells of these mice. HBV antigen specificity was demonstrated by a 19-fold greater response for X-SCore than Yvec-immunized mice.

Adoptive transfer tumor protection

Tumor challenge studies were conducted to determine if HBV Tarmogens generate protective responses in vivo. In the first model, splenocytes from vaccinated mice were transferred to SCID mice, which were then challenged with 30,000 EL4 tumor cells expressing the S antigen. The results showed that immune cells from X-SCore vaccinated mice significantly inhibited growth of the EL4 tumors relative to Ovax. This demonstrates HBV antigen-specific, immune-mediated protection in vivo with X-SCore/Ovax. The second model was performed using SCID mice infected with 5,000 EL4 tumor cells expressing either the X or S antigen. X-SCore vaccination significantly inhibited growth of the EL4 tumors relative to Ovax. This demonstrates HBV antigen-specific, immune-mediated protection in vivo with X-SCore/Ovax.

Prophylactic tumor protection in immunocompetent mice

In a second model, C57BL/6 mice were vaccinated both with three weekly HBV Tarmogen vaccinations, challenged one week later with 300,000 HBV EL4 tumor cells, and tumors were palpated daily for 21 days. The results showed that both HBV Tarmogen significantly inhibited tumor growth relative to the Ovax control. (Fig 6a, tumor size; b, Kaplan-Meier survival analysis) again showed X-SCore antigen-specific protection in vivo.

Conclusions

• The first HBV Tarmogen is undergoing a clinical translation path, navigating clinical trials with a goal of therapeutic vaccine for chronic HBV infection

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Active immunotherapy with yeast-based Tarmogens

Administration of Tarmogens initially results in binding of the yeast to antigen-presenting cells, the most important of which are dendritic cells, near the injection site. The dendritic cells are activated as a result of the Tarmogens binding to Toll-like receptors and other receptor molecules on the surface of the dendritic cell, resulting in the activation of cytokine immune signaling molecules.

The Tarmogen is processed by the dendritic cell in two ways. First, the Tarmogen is engulfed by endosomes and the protein inside the endosome is cut into shorter peptides fragments. These peptides are presented by Class II MHC molecules on the surface of the dendritic cell. In combination with IL-12, a cytokine that is produced by the dendritic cell, these MHC-peptide complexes on the surface of the dendritic cell are recognized by and activate cells involved in viral immunity called CD4+ helper T cells.

Dendritic cells also process Tarmogens by engulfing them with phagosomes. This results in presentation of peptides, including the antigen from inside the Tarmogen, to CD8+ killer T cells. CD4+ helper T cells are so named because one of their roles is to "help" activate killer T cells by expressing interferon gamma (IFNγ).

The newly activated CD8+ killer T cells move throughout the body and identify any other cell that expresses the same disease protein as the one recognized by the CD8+ killer T cells. Once the CD8+ killer T cell finds another cell in the body containing the target protein, it can kill the cell using multiple mechanisms.

Recombinant yeast therapeutic vaccines expressing hepatitis B virus (HBV) X, S, and Core antigens generate antigen specific T cell responses and tumor protection in mice

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Purpose: Chronic HBV is characterized by suboptimal T cell responses to viral antigens. A therapeutic vaccine capable of restoring the immune response to HBV would be an attractive clinical option. A yeast-based immunotherapy platform (Tarmogens) was used to make product candidates expressing HBV X, S, and Core antigens. One version contains a chimera of HBV S and Core (SCore), and another contains a chimera of HBV X, S and Core (XSCore). These were used to elicit antigen specific T cell responses and protection from challenge with HBV S and Core expressing tumors.

Methods: Mice were subcutaneously immunized with 3 weekly doses of SCore, XSCore, or empty yeast (ctrl). One week later immune cells were evaluated by lymphocyte proliferation, interferon g (IFNγ)/IL2 ELISpot, and intracellular cytokine staining assays to assess T cell responses. Vaccinated mice were also challenged by EL4 cells expressing HBV S and Core antigens and tumor protection was evaluated with a C3H mice model.

Results: Mice immunized with SCore and XSCore compared to controls showed induction of lymphocyte proliferation (32 fold increase vs. ctrl), IFNγ (19-fold increase), IL2 (5.3-fold increase), and TNF-a (1.6-fold increase) when stimulated by recombinant HBV antigens and with HBV peptides associated with acute HBV clearance. CD4+ and CD8+ responses were observed. Mice vaccinated before tumor challenge showed delayed growth of EL4 cells expressing HBV antigens over an 11 day period. At day 7 post challenge, 100% of SCore and 90% of XSCore treated mice were tumor free while 44% of ctrl mice were tumor free at this time point. At 7 days post challenge showed improved survival (33% progression free for XSCore vs. 0% for control, day 18).

Conclusions: SCore and XSCore elicit HBV specific T cell activation and protection against challenge by HBV antigen expressing tumors, suggesting that these Tarmogens could be used to improve HBV S antigen seroconversion in chronic HBV patients.