TARGETING INFLUENZA WITH YEAST-BASED TARMOGEN IMMUNOTHERAPY

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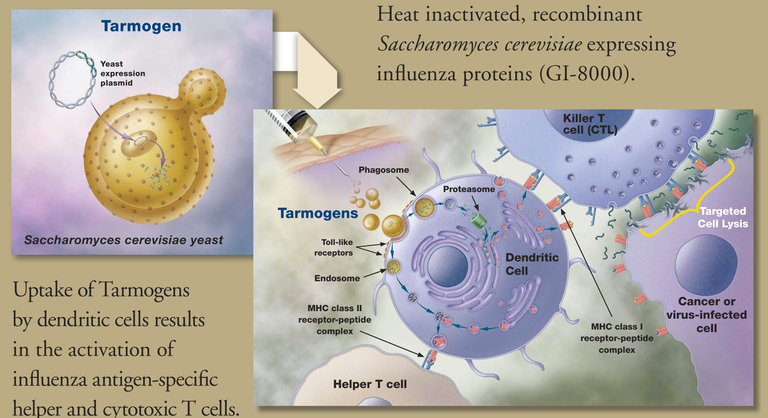
Tarmogen Technology



Uptake of Tarmogens by dendritic cells results in the activation of influenza antigen-specific helper and cytotoxic T cells.

Abstract

Administration of whole recombinant *S. cerevisiae* yeast engineered to express heterologous disease antigens (Tarmogens™) elicits potent CD4+ and CD8+ T cell responses. Preclinical studies have shown that the magnitude of the immune responses that are induced is dependent on the number of yeast per dose, the number of doses administered, and the quantity of target antigen per yeast. Since neutralizing anti-yeast responses were not observed, even after repeated administration, Tarmogens can be used for priming and boosting of the immune response. Two products are currently in human clinical trials: GI-4000 expressing mutated Ras antigen for the treatment of cancer, and GI-5005 expressing HCV NS3 and Core antigens targeting patients with chronic hepatitis C infection.

Tarmogens expressing HIV antigens that are located behind the yeast cell wall induced antibody titers in animals upon subsequent exposure to soluble antigen. Thus, the yeast-based approach may be used to prime helper T cells and trigger B cell responses in order to generate cytotoxic T cell as well as antibody responses. Yeast expressing conserved influenza virus proteins (e.g., matrix, nucleoprotein) in the cytoplasm or hemagglutinin on the cell surface are being tested in mice for induction of influenza-specific cellular immune responses, neutralizing antibodies, and protection from influenza challenge. This non-egg-based approach may enable rapid, large scale vaccine production to meet demand in case of influenza epidemics.

Introduction

Tarmogens (targeted molecular immunogens) are whole, heat-killed recombinant *Saccharomyces cerevisiae* yeast engineered to express one or more target protein antigens, and activate both an innate immune response via toll-like receptors (TLRs), as well as an adaptive antigen-specific immune response. Tarmogens can also prime T Helper cell responses efficiently to facilitate antibody production by B cells. In order to generate a cell-based influenza vaccine, influenza proteins were expressed in *S. cerevisiae* and evaluated for their ability to induce both cellular immune responses as well as antibodies. GI-8000 $M1_{IC}$ expresses the matrix protein of influenza in the cytosol of the yeast. The influenza matrix protein was chosen as target because it is highly conserved, contains epitopes recognized by both $CD4^+$ and $CD8^+$ T cells, and induces cross-protective cytotoxic T cell responses. The influenza hemagglutinin protein induces protective substrain-specific neutralizing antibodies. Tarmogens engineered to express the HA protein on the yeast surface (GI-8000 HA_{SF}) as well as in the cytosol (GI-8000 HA_{IC}) is currently under evaluation for eliciting neutralizing antibodies that can protect from wild type virus challenge in pre-clinical animal models.

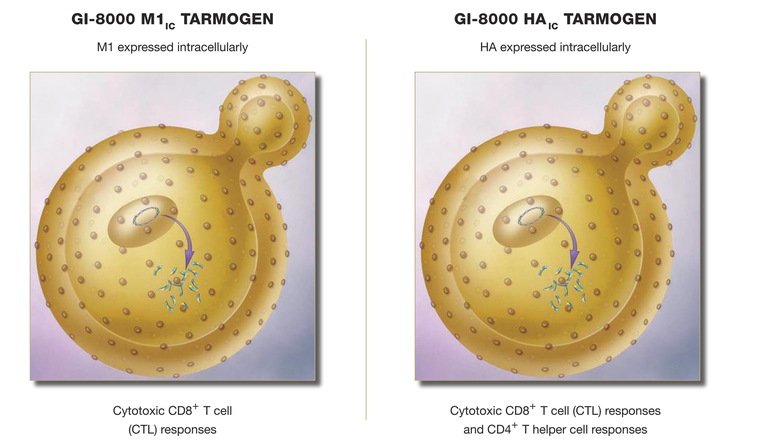


Figure 1. Tarmogens have been created to intracellularly express the conserved influenza protein M1 (GI-8000 MI_{IC}) and the hemagglutinin surface protein (GI-8000 HA_{IC}) in the cytosol.

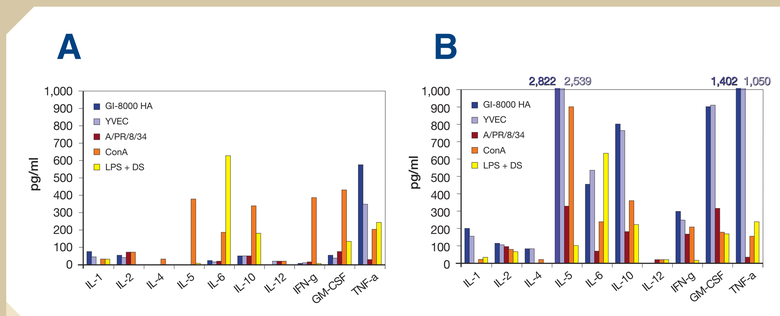


Figure 2. Cytokine profiles from GI-8000 HA_{IC}-immunized mice

BALB/c mouse splenocytes that were derived from naïve (A) or immunized mice (B), were stimulated *in vitro* with GI-8000 HA_{IC}, YVEC (empty yeast vector), inactivated A/PR/8/34, Conacavalin A (Con A), or Lipopolysaccharide (LPS) and Dextran sulfate (DS). On day 3, supernatants were harvested and analyzed for cytokines present.

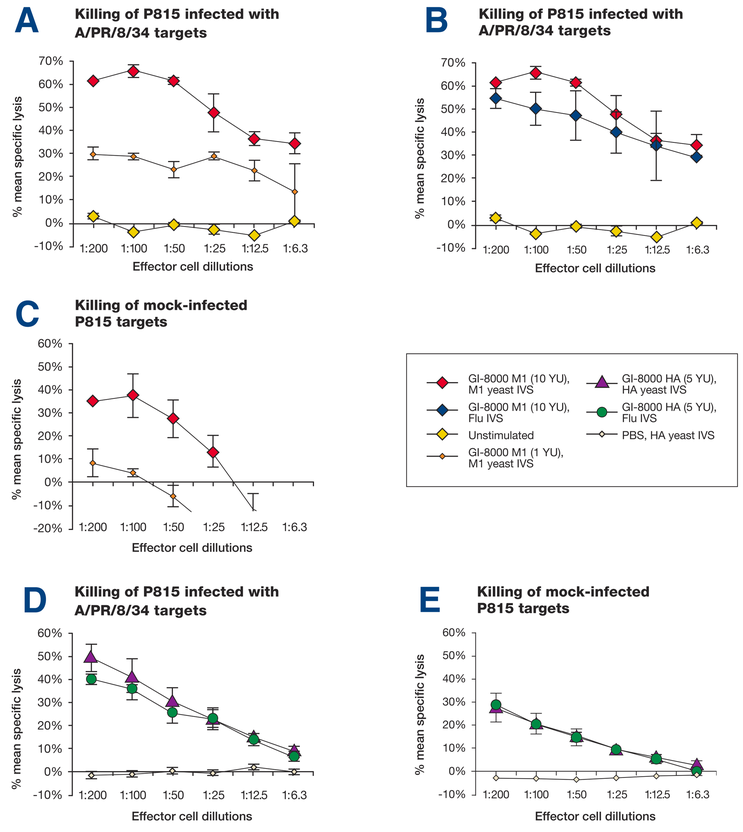


Figure 3. Dose-dependent induction of Influenza-specific CTL with GI-8000 $M1_{IC}$ and GI-8000 HA_{IC} yeast

Spleen cells from BALB/c mice that were immunized subcutaneously weekly for three weeks with 1 or 10 YU GI-8000 $M1_{IC}$ (A, B, C) or with 5 YU GI-8000 HA_{IC}, were tested for their ability to kill syngeneic P815 target cells (A, B, D) infected with A/PR/8/34 influenza virus or mock-infected P815 target cells (C, E) in a standard chromium release assay.

YU = Yeast unit (1 YU = 1 x 10^7 cells). Results are expressed as the mean +/- S.D. for triplicate samples.

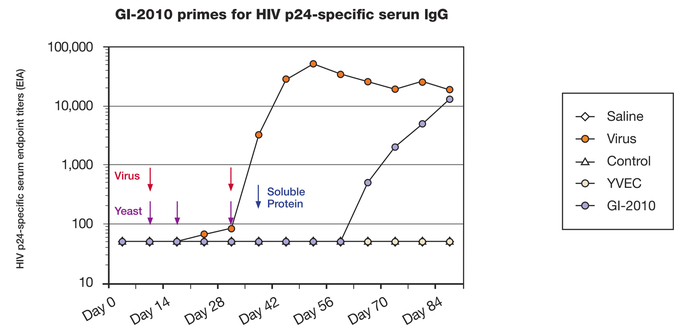


Figure 4. HIV Tarmogen priming of T Helper cells and induction of gag antibodies

Macaques immunized twice with GI-2010, a Tarmogen expressing the HIV gag protein, display high levels of gag antibodies after boosting with soluble protein.

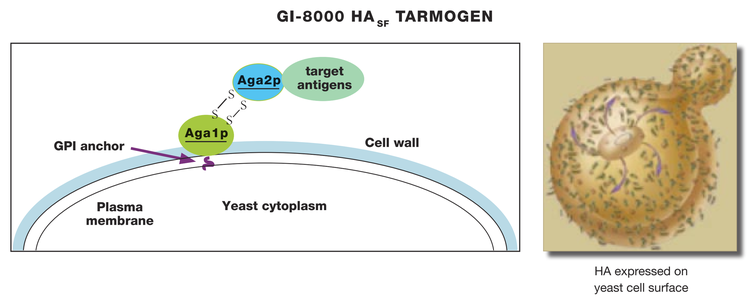


Figure 5. Yeast cell surface antigen expression system for influenza HA protein

The surface Aga2-fusion antigen is soluble and accessible for antibody and ligand binding. The non-covalent disulfide linkages between Aga1p and Aga2p affords easy release of the surface antigen. Approximately 30,000 molecules can be displayed per cell. GI-8000 HA_{SF} is currently being evaluated for induction of neutralizing antibody and protection from wild type influenza challenge in a mouse model.

Conclusions

- Yeast represent a cost-effective, cell-based influenza vaccine approach
- GI-8000 Tarmogens induce both Th-1 and Th-2 type cytokines
- GI-8000 induces dose-dependent, influenza-specific cytotoxic T cell responses
- Tarmogens prime T Helper cell responses thereby activating B cells
- Studies evaluating antibody induction in mice by GI-8000 (HA surface expression) and protection from wild type influenza challenge are ongoing