

P5 Genetic Immunization Using the *flaA* Gene Confers Partial Protection Against *Helicobacter pylori* Infection in Germ-Free C57/BL6 Mice

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Objective: To determine if genetic immunization using a gene required for motility (*flaA*) protects germ-free mice against *Helicobacter pylori* infection.

Methods: The *flaA* gene from *H. pylori* strain N6 was PCR amplified and ligated into the plasmid pVR1020 (provided by Vical, Inc.). For vaccination, three groups of five germ-free C57/BL6 mice received either tris-buffer alone, 85-100 µg of pVR1020 without insert, or 85-100 µg of pVR1020-*flaA*, distributed over four sites intramuscularly. The vaccination was repeated after 45 days and mice were challenged one month later with 3×10^8 CFU of *H. pylori* strain SS1. Three weeks post-challenge mice were sacrificed and CFU/g of stomach tissue was determined. Severity of gastritis was determined by quantification of the percent gastric mucosa with neutrophilic adenitis.

Results: Buffer-vaccinated mice were colonized with $2.54 \pm 1.29 \times 10^6$ CFU/g. Mice vaccinated with pVR1020 were colonized with $3.02 \pm 0.37 \times 10^6$ CFU/g. Mice receiving pVR1020-*flaA* were colonized with $9.10 \pm 2.72 \times 10^5$ CFU/g. Mice vaccinated with pVR1020-*flaA* had an approximately three-fold reduction in colonization compared to mice vaccinated with pVR1020 without insert ($p < 0.0001$). Gastritis was more severe ($11.0 \pm 6.4\%$ gastric mucosa) in *flaA* vaccinated mice than in mice vaccinated with either buffer ($4.2 \pm 5.8\%$) or vector alone ($2.0 \pm 3.5\%$), $p=0.02$.

Discussion: These results demonstrate the potential of genetic immunization as a means of preventing *Helicobacter* infection. Complete protection may be possible through the enlistment of other motility associated genes in this vaccination regimen. The slightly increased inflammation in vaccinates compared with unvaccinated, infected animals is similar to that seen in conventionally vaccinated mice, and most likely represents the local immune response responsible for partial protection.

P7 Effective Induction of Immune Response to Japanese Cedar Pollen Allergen (Cry j 1) in Mice by Gene Gun DNA Delivery System

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Japanese cedar (*Cryptomeria japonica*) pollinosis is one of the common immediate-type allergic diseases in Japan. To establish immunotherapy for cedar pollinosis, we had a plan to utilize the method of DNA vaccination. We constructed plasmid DNA encoding Cry j 1 (pCACJ1), a major Japanese cedar pollen allergen, which permitted the expression in mammalian cells *in vitro*. Repeated immunization of BALB/c mice intradermally with 1.0 µg of pCACJ1 by gene gun caused significant anti-Cry j 1 antibody response, which was predominated by IgG1 antibodies. A single challenge with alum-precipitated Cry j 1 into pCACJ1-immunized mice induced production of anti-Cry j 1 IgE antibodies, significantly higher than that observed in non-immunized mice, compatible with the notion that pCACJ1 immunization by gene gun primed Cry j 1-specific B cells, resulting into class switch from μ to ϵ .

Splenic T cell isolated from pCACJ1 immunized mice proliferated in response to Cry j 1 *in vitro* as well as a peptide corresponding to a dominant T cell epitope in Cry j 1. In addition, spleen cells isolated from the immunized mice secreted IL-4 *in vitro*, but not IFN- γ , upon stimulation with Cry j 1. Taken together, these data suggest that pCACJ1 immunization by gene gun caused a Th2 response to Cry j 1. The effect of pCACJ1 injection into muscle in anti-Cry j 1 IgE response, which could elicit Cry j 1-specific Th1 response, is now under investigation.

P6 Advantages of Using Novel Carrier Proteins in Polysaccharide Conjugates

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Many of the polysaccharide conjugate vaccines which have been licensed for use or are currently in clinical trials have used proteins previously found safe in humans. However, investigators are now concerned about the over use of these standard carriers and in some instances found them detrimental in their vaccine formulation. We have investigated in pre-clinical studies the use of several novel carrier proteins for each of the individual polysaccharide conjugate vaccines currently under development: *N. meningitidis* capsular polysaccharide, Group B streptococcal capsular polysaccharide, *H. influenzae* capsular polysaccharide, and pneumococcal capsular polysaccharide. We have selected a protein in each case that would not only supply the carrier function but would also contribute to the vaccine in other ways, either by greatly enhancing the immune response to the polysaccharide or by replacing one of the polysaccharides in a multivalent formulation. We have found that the porin protein used in the meningococcal capsular polysaccharide conjugate vaccine to be an exciting immuno-enhancing protein which greatly increases the effectiveness of this vaccine and other polysaccharides to which it is conjugated. The C β protein of GBS was able to reduce the number of polysaccharides used in this multivalent vaccine as well as to reveal a novel means of killing GBS bacteria. The pneumolysoid used as the carrier for the pneumococcal polysaccharide conjugates will also be presented.

P8 Application of Antigen-Liposome Conjugates to Vaccine Development

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In the previous study, we have reported that ovalbumin (OVA) coupled with liposomes induces anti-OVA IgG but not anti-OVA IgE antibody production. The antigen-liposome conjugate was expected to be used in a novel protocol of vaccination that induces the least IgE antibody production.

In this study, tetanus toxoid (Ttd)-liposome conjugate was made and the induction of antibody production and protection against subsequent challenge with tetanus toxin (Ttx) were investigated in mice. The Ttd-liposome conjugate induced a significant anti-Ttd IgG antibody production with least anti-Ttd IgE antibody production and successfully induced protection against challenge with lethal dose of Ttx. Similar results were obtained when Shiga-like toxin (SLT) was coupled with liposome. The SLT-liposome conjugate successfully induced protection either against challenge with SLT or against oral infection with O157:H7 in mice. Thus, these results suggested that antigen-liposome conjugates may possess a potential ability to serve as a novel protocol for vaccination which induces production of protective antibodies without IgE synthesis.