

New Perspectives for the Treatment of Food Allergy (Peanut)

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Introduction

Food allergy affects about 6%–8% of children less than 4 years of age and about 2% of the U.S. population beyond the first decade of life [1], and is the single leading cause of anaphylaxis treated in hospital emergency departments in the U.S. and many “westernized” countries. Extrapolating from a regional survey in Olmstead County, Minnesota [2], it is estimated that food allergy accounts for about 30,000 anaphylactic reactions, 2000 hospitalizations and 200 deaths each year in the United States. Peanut and tree nut allergy account for the vast majority of fatal and near-fatal anaphylactic reactions [3,4]. A national survey indicated that about 1.1%, or 3 million Americans, are allergic to peanuts and/or tree nuts [5]. Ironically, despite an increasing public awareness of food allergy, most patients are ill-prepared to deal with anaphylactic reactions [6]. In a recent series, over 80% of patients dying from food allergic reactions were not provided with appropriate information or self-injectable epinephrine to avoid or manage accidental food-induced reactions [4].

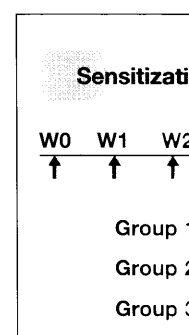
Unlike traditional immunotherapy for inhalant and bee sting allergy, the benefit-to-risk ratio for injections of peanut extracts appears to be unacceptable. In a trial of standard rush immunotherapy in 6 patients with peanut allergy, Nelson and colleagues were able to achieve “maintenance” levels in 4 of 6 patients, but in 2 subjects the dose had to be reduced because of unacceptable side effects [7]. While the 4 patients achieving “maintenance” therapeutic doses were able to tolerate significantly more peanut during subsequent blinded challenge compared to 6 placebo control subjects, this beneficial effect was lost in 2 patients when the allergen dose had to be decreased due to adverse side effects. Overall, the adverse reactions rate was 23% during the “build-up” phase and 39% during the “maintenance” phase. Given this unfavorable benefit-to-risk ratio, several novel immunotherapeutic strategies are being examined as treatment modalities for food allergy: (1) humanized anti-IgE monoclonal antibody therapy, (2)

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“engineered” (mutated) allergen protein immunotherapy, (3) antigen-immunostimulatory sequence-modulated immunotherapy, (4) peptide immunotherapy, (5) plasmid-DNA immunotherapy, (6) cytokine-modulated immunotherapy, and (7) traditional Chinese medicine [8]. Administering monthly injections of humanized recombinant anti-IgE antibodies, which reduces mast cell- and basophil-bound IgE, appears to be effective in preventing allergic responses in peanut-sensitive subjects, at least to small amounts of peanut protein [9]. Our group has focused on another approach utilizing “engineered” (mutated) recombinant peanut proteins, which contain substitutions of critical amino acids within the IgE-binding epitopes, to prevent activation of IgE-mediated reactions seen in “traditional” desensitizing immunotherapy.

In collaboration with Drs. Burks and Bannon, we have developed engineered major allergenic proteins to eliminate IgE binding but retain T cell proliferation. It was felt that these proteins could be injected safely without eliciting an allergic response, but perhaps more importantly, they would not be taken-up by IgE-bearing antigen-presenting cells (APCs) and therefore would not augment a Th2 response. The three major peanut allergens (Ara h 1, Ara h 2, and Ara h 3) were identified, isolated, sequenced, and the sequential IgE-binding epitopes mapped, and the DNA encoding these proteins was cloned. Critical amino acids for IgE binding to allergenic epitopes were determined by single amino acid substitutions of peptides generated on a nitrocellulose matrix (Genysis SPOTs membranes), and then recombinant proteins were “engineered” to eliminate the IgE-binding epitopes. Using site-directed mutagenesis of the allergen cDNA clones followed by recombinant production of the modified allergen, engineered DNA was developed that coded for proteins that differ by a single amino acid within each of the IgE-binding epitopes [10]. Overall, 23 binding sites (epitopes) were modified in Ara h 1, 10 binding sites in Ara h 2, and 4 sites in Ara h 3. The engineered recombinant proteins had markedly reduced IgE antibody binding utilizing sera from peanut-allergic patients, but promoted comparable T cell proliferation as seen with the native peanut proteins.

Methods and Results

In an attempt to study novel immunomodulatory therapies, we developed a mouse model of peanut anaphylaxis using intragastric sensitization and challenge, which closely reflects the clinical characteristics of peanut allergy in man [11]. Peanut-induced anaphylaxis in this model is IgE-mediated, and mast cell degranulation and histamine release were shown to be associated with the anaphylactic symptoms. We further

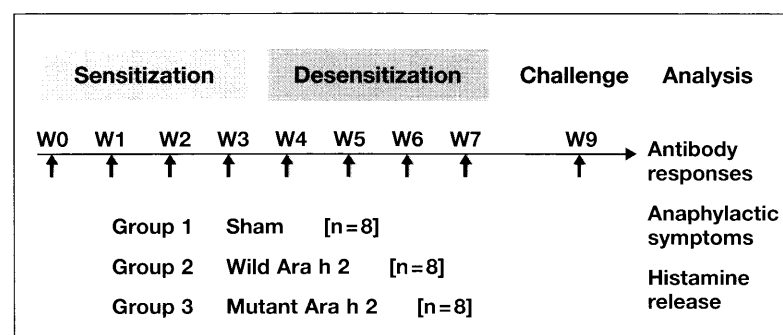


Fig. 1.
Comparison of
native versus
“engineered”
Ara h 2:
Experimental
protocol.

demonstrated that B cell and T cell responses of peanut-allergic mice were similar to those observed in peanut-allergic man. This model appears to closely mimic physiological and immunological phenomena seen in human peanut-allergic patients.

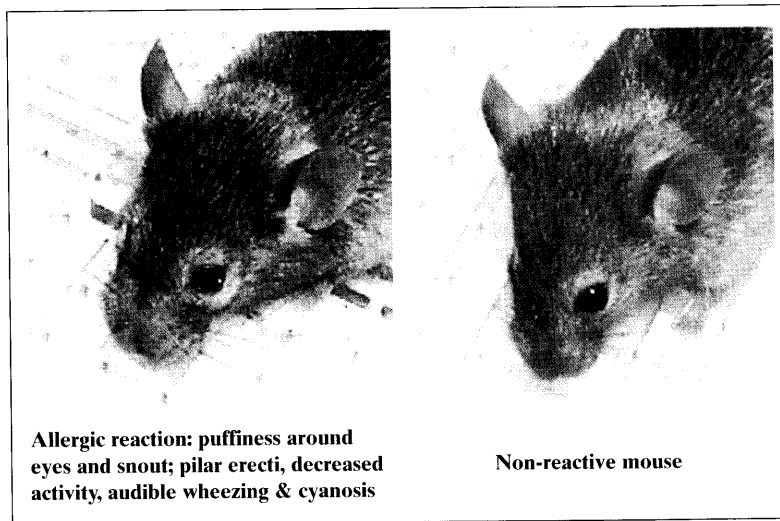


Fig. 2. Allergic reaction in peanut-sensitized mice: score = 3.

As depicted in Figure 1, 5-week old C3H/HeJ mice were sensitized to Ara h 2 and then treated with four weekly doses of either native-Ara h 2 (n-Ara h 2) or engineered, recombinant-Ara h 2 (r-Ara h 2) injected subcutaneously. Mice were then challenged with Ara h 2 and their reactions scored utilizing a scoring system previously published: scored as follows: 0 - no symptoms; 1 - scratching and rubbing around the snout and head; 2 - puffiness around the eyes and snout, pilar erecti, decreased activity and/or increased respiratory rate; 3 - audible wheezing, labored respiration, cyanosis around the snout, ears, feet and tail; 4 - no activity after gentle prodding, or tremor and con-

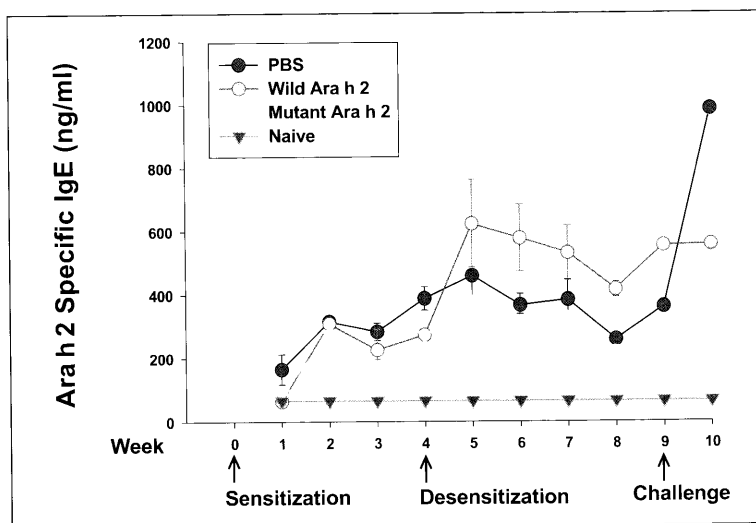


Fig. 3A: Ara h 2-specific IgE levels following immunotherapy with n-Ara h 2 (wild) and r-Ara h 2 (mutant).

vulsion; 5 - death demonstrated no difference compared to mice sensitized with n-Ara h 2 except for (untreated) controls receiving the r-Ara h 2 treated with PBS. Mice challenged were sensitized to Ara h 2 (data not shown).

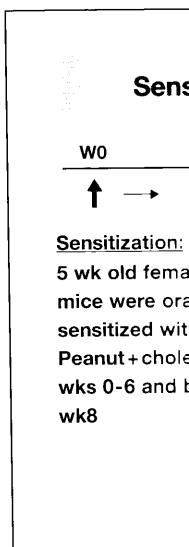
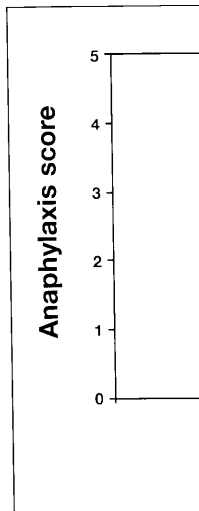


Fig. 4. Protocol for intragastric or rectal

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patients.

vulsion; 5 – death (Figure 2). As shown in Figure 3A, mice treated with r-Ara h 2 demonstrated no rise in their Ara h 2-specific IgE levels following immunotherapy compared to mice treated with n-Ara h 2 (conventional) therapy. While mice treated with n-Ara h 2 experienced slightly less severe allergic reactions than their PBS-treated (untreated) counterparts, the clinical difference was not appreciable (Figure 3B). Mice receiving the r-Ara h 2 had a marked reduction in clinical symptoms compared to mice treated with PBS or n-Ara h 2 (Figure 3B). Plasma histamine levels following peanut challenge were significantly lower than PBS-treated mice only in mice treated with r-Ara h 2 (data not shown).

Fig. 2. Allergic reaction in peanut-sensitized mice; score = 3.

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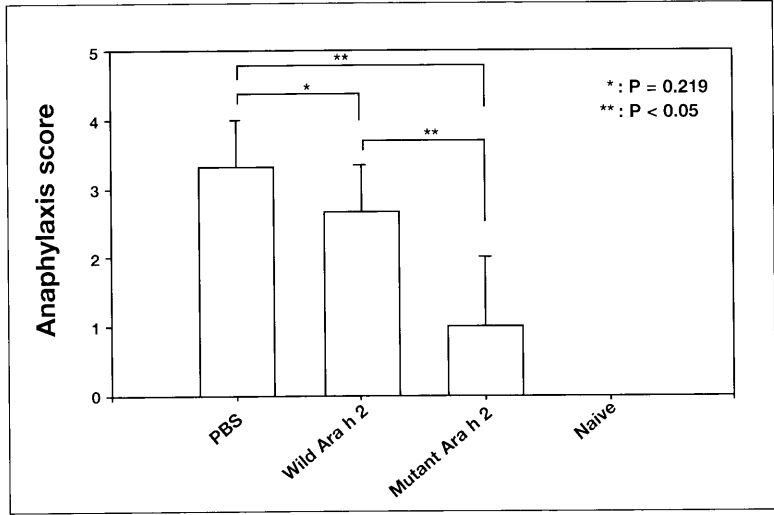


Fig. 3B. Anaphylaxis scores following oral challenge with Ara h 2 in mice treated with n-Ara h 2 (wild) or r-Ara h 2 (mutant).

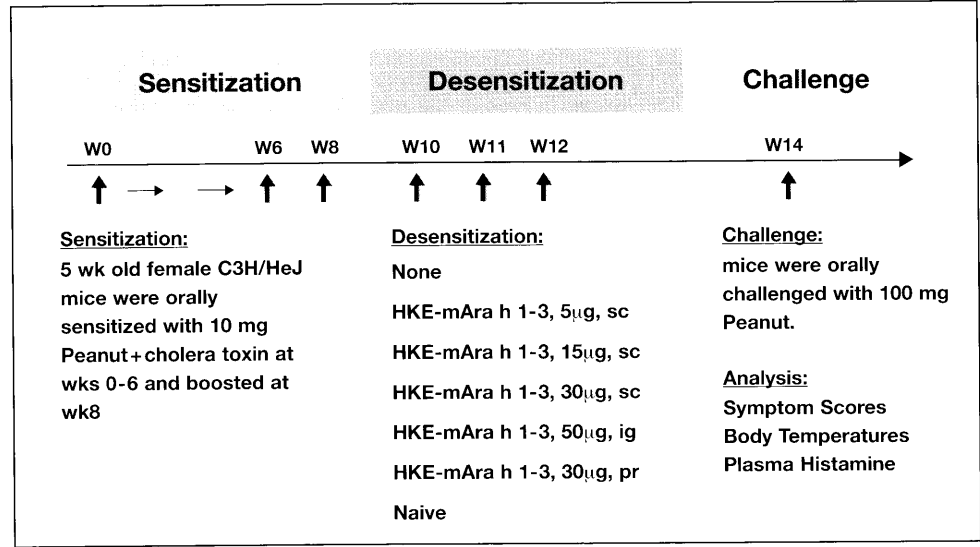


Fig. 4. Protocol investigating the efficacy of HKE-m-Ara h 1-3 administered by the subcutaneous, intragastric or rectal routes.

Fig. 3A: Ara h 2-specific IgE levels following immunotherapy with n-Ara h 2 (wild) and r-Ara h 2 (mutant).

Several investigators have shown that the use of antigen linked to cytokines (e.g. IL-12, IL-18) [12] or the use of bacterial adjuvants, such as heat-killed *Listeria monocytogenes* [13], could induce deviation of antigen-specific Th2 responses to more Th1-like responses. Since our engineered recombinant proteins are generated in *E. coli*, and since *E. coli* possess lipopolysaccharides that favor Th1 responses, we hypothesized that administering heat-killed *E. coli* containing the purified r-Ara h 1-3 proteins may prove more efficacious than administering modified Ara h 1-3 proteins alone. Subsequent experiments demonstrated that administering heat-killed *E. coli* containing r-Ara h 1-3 proteins (HKE-Ara h 1-3) subcutaneously or per rectum were more effective at "desensitizing" peanut-allergic mice, as determined by post-challenge clinical score (Figures 4 and 5), body temperature, airway response and plasma histamine compared to the PBS control (sham-treated) mice or mice receiving HKE-mAra h 1-3 administered by the intragastric route. Peanut-specific IgE was significantly decreased in mice treated with HKE-mAra h 1-3 administered by the subcutaneous or rectal routes, but this decrease was most marked in the mice receiving rectal HKE-mAra h 1-3 (data not shown). Following the oral challenge, spleen cells were isolated and cultured in the presence or absence of peanut protein. As demonstrated in Figure 6, spleen cells from mice receiving HKE-mAra h 1-3 were significantly suppressed compared to cells from PBS-treated (sham) mice. In addition, splenic cells from these mice cultured in the presence of peanut protein produced significantly less IL-4 and IL-5 compared to PBS-treated mice or mice receiving HKE-mAra h 1-3 administered by the intragastric route; no difference in peanut-induced interferon-gamma production was seen among any group (data not shown).

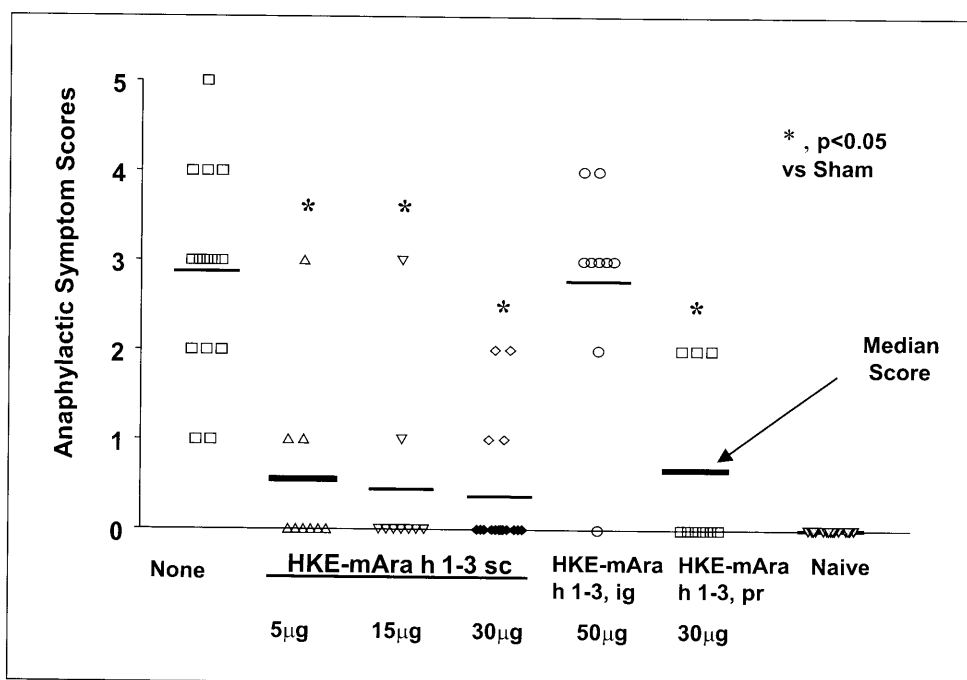


Fig. 5. Symptoms scores following desensitization with HKE-mAra h 1-3 [from Li et al. manuscript in preparation].

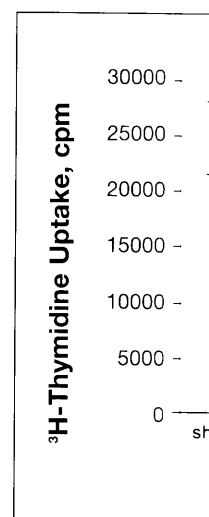


Fig. 6. Spleen cell proliferation.

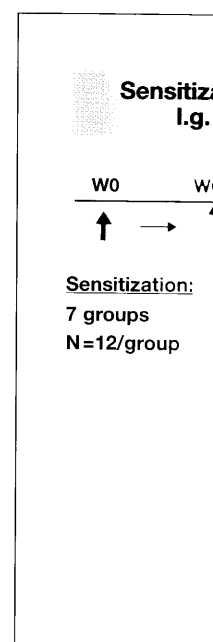


Fig. 7. Protocol comparing desensitization at different doses.

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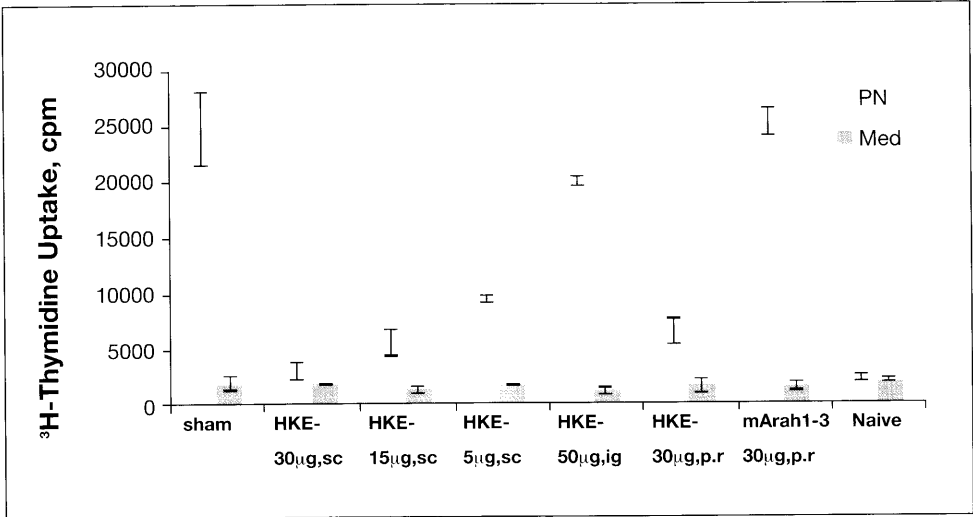
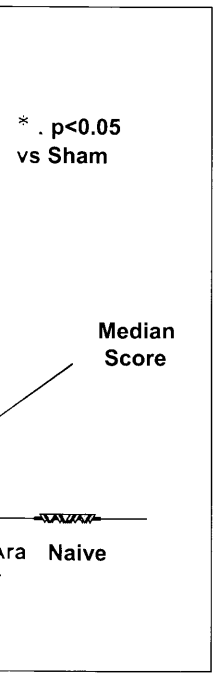


Fig. 6. Spleen cell proliferation following desensitization with HKE-mAra h 1-3.

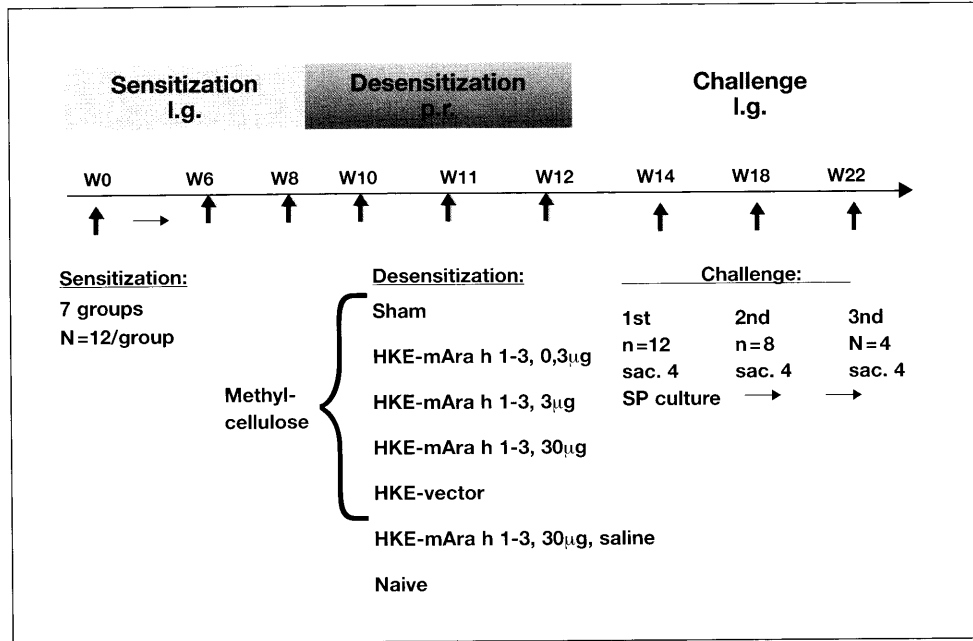


Fig. 7. Protocol comparing desensitization with HKE-mAra h 1-3 administered rectally at different doses in methylcellulose or saline.

Given the strong “desensitizing” effect of administering HKE-mAra h 1-3, given the potential adverse side effects of administering a whole cell vaccine subcutaneously, and given the fact that the rectal mucosa is heavily colonized with *E.coli* and hundreds of other organisms, it was decided to further investigate the rectal route of vaccine administration. In the next set of experiments, different doses of HKE-mAra h 1-3 were

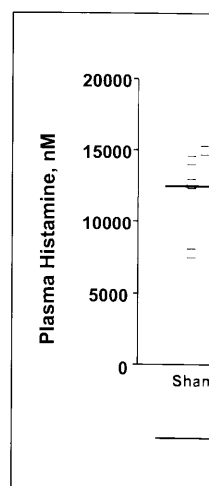
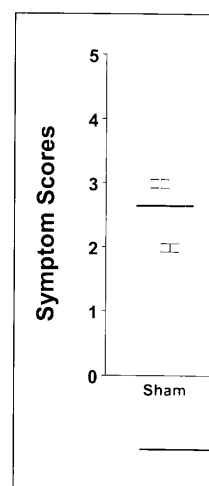
administered in a methylcellulose carrier (to simulate a suppository form of the drug in man) or saline and then challenged one month, two months and three months following the third dose of rectal HKE-mAra h 1-3 (Figure 7). A portion of each group of mice was sacrificed after each challenge to determine the effect of the desensitization protocol on peanut-induced splenic cell proliferation and cytokine production. Figure 8 depicts the outcome of the third peanut challenge performed 12 weeks after the final HKE-mAra h 1-3 administration. As can be seen, only mice receiving the 3 μ g and 30 μ g doses of HKE-mAra h 1-3 in methylcellulose were significantly protected at 12 weeks post-therapy, and only the 30 μ g treated group had significantly lower plasma histamine at this time (Figure 8A and 8B). Interestingly, all HKE-mAra h 1-3 groups were protected after the first challenge (week 14). However, the 0.3 μ g HKE-mAra h 1-3 in methylcellulose and the 30 μ g HKE-mAra h 1-3 in saline just failed to reach clinically significant protection following the second challenge (week 18) but were not significantly different than PBS in preventing symptoms after the third challenge (week 22).

Discussion

The numbers of severe and fatal food allergic reactions indicate that the current methods of therapy, i.e. allergen avoidance, are not adequate for dealing with this disorder. In addition, standard immunotherapeutic approaches also are not adequate due to their unacceptably high risk-to-benefit ratio. Novel therapeutic approaches are clearly needed for the treatment of food allergy. In the past several years, we have evaluated the efficacy of "engineered" recombinant proteins in our mouse model of peanut-induced anaphylaxis. Allergenic (IgE-binding) epitopes were modified by a single amino acid substitution in order to largely abrogate IgE binding to the proteins (mAra h 1-3) for use in the vaccine. It was felt that this would decrease the risk of inducing an allergic response following the administration of the protein in peanut-allergic subjects and largely eliminate stimulation of Th2 responses that are promoted by IgE-bearing APCs in allergic patients, and which are likely to interfere with the achievement of desensitization in many cases. Administration of mAra h 2 was shown to be superior to nAra h 2 in the "desensitization" of Ara h 2-allergic mice (Figure 2).

Work by others suggested that administration of allergenic proteins with a bacterial adjuvant may be more efficacious in "down-regulating" the allergic response [13]. Since our modified Ara h 1-3 proteins are produced in *E. coli* that do not produce endotoxin, it was decided to investigate the adjuvant effect of heat-killed *E. coli* producing the three modified peanut proteins (HKE-mAra h 1-3) in "down-regulating" the allergic response in peanut-sensitized mice. In addition to the possible adjuvant effect, it was felt that administering the mAra h 1-3 proteins within *E. coli* would provide another level of protection by preventing the modified proteins from activating mast cells. As shown in Figure 5, HKE-mAra h 1-3 administered subcutaneously or per rectum was very effective in protecting peanut-allergic mice. In other studies not shown, HKE-mAra h 1-3 was shown to be more effective than mAra h 1-3 alone injected subcutaneously. Administration of HKE-mAra h 1-3 led to a decrease in serum peanut-specific IgE, slight rise in peanut-specific IgG2a, a decrease in peanut-induced splenic T cell proliferation and IL-4 and IL-5 production *in vitro*, and no significant change in peanut-induced splenic T cell interferon-gamma production *in vitro*.

Given the apparent protective effect of the vaccine alone, but the potential for systemic allergic reactions, it was decided to evaluate the vaccine administered rectally. As shown in Figure 6, the methylcellulose carrier was not significantly different compared to other formulations. The rectal administration of heat-killed *E. coli* is beneficial in the absence of the bacterial organisms. The rectal route of administration of the vaccine administered rectally was not significantly different from the protective effect of the vaccine administered rectally. In the future, peanut-allergic subjects may benefit from a suppository-like form of the vaccine.



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Given the apparent improved efficacy of HKE-mAra h 1-3 compared to mAra h 1-3 alone, but the potential for adverse side-effects of HKE-mAra h 1-3 injected subcutaneously, it was decided to further investigate the potential of HKE-mAra h 1-3 administered rectally. As demonstrated in Figure 8, administration of HKE-mAra h 1-3 in a methylcellulose carrier provided sustained protection in peanut-sensitized mice compared to other forms of administration. Based on these studies, it appears that the rectal administration of HKE-mAra h 1-3 could provide a number of advantages: the heat-killed *E. coli* is being administered into an environment replete with *E. coli* and other bacterial organisms which should lessen concerns about safety of such administration, the rectal route of HKE-Ara h 1-3 required fewer doses of the engineered proteins than the vaccine administered by the subcutaneous route [Li et al; in preparation], and the protective effect appeared to be more long lasting. These studies suggest that in the future, peanut-allergic patients may be successfully "desensitized" utilizing a suppository-like form of HKE-mAra h 1-3.

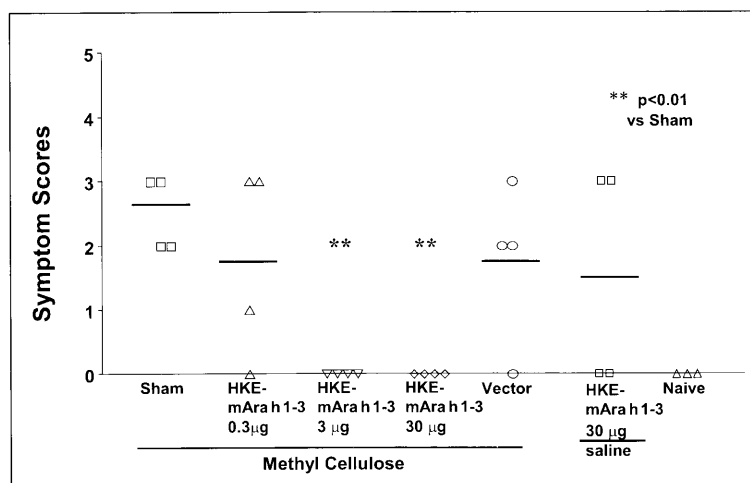


Fig. 8A.
Symptom scores following 3rd oral peanut challenge 3 months after the last dose of HKE-mAra h 1-3 (week 22) administered rectally [from Li et al. manuscript in preparation].

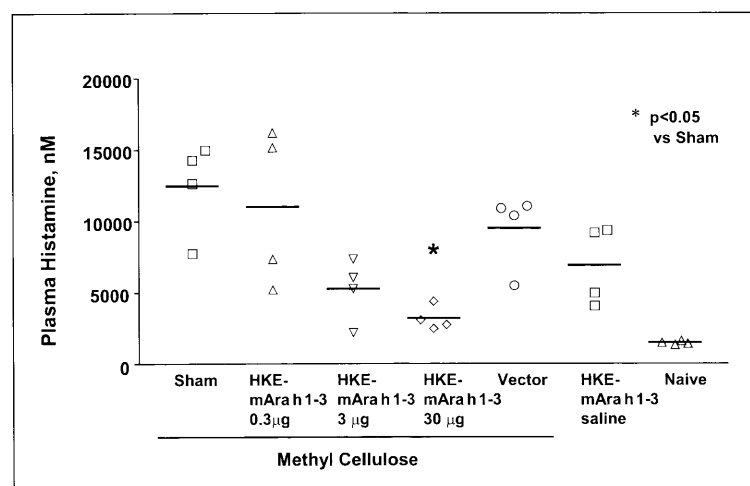


Fig. 8B.
Plasma histamine levels following 3rd oral peanut challenge 3 months after the last dose of HKE-mAra h 1-3 (week 22) administered rectally [from Li et al. manuscript in preparation].

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13. Yeung VP, Gieni RS, Umetsu DT, DeKruyff RH. Heat-killed *Listeria monocytogenes* as an adjuvant converts established murine Th2-dominated immune responses into Th1-dominated responses. *J Immunol* 1998; 161: 4146-52.

Discussion

Ferreira: Did you have any control as to the load and exposure of the bacteria using the rectal route of treatment?

Sampson: Yes, basically we tried to calculate equivalents. So when we administered 30 μg or 3 μg of protein, we basically calculated how much protein will be present in the bacteria if we tried to isolate the protein. We used that equivalent amount of bacteria for administration.

Petrunov: I would like to ask you what kind of animals have you used in your experiments? I mean germ-free or conventional animals? If you used conventional animals you know very well that these animals are colonized with many different gram negative bacteria which contain a lot of endotoxin. Endotoxin is well known as a polyclonal stimulator of the immune system and endotoxin may interfere with your results. Would you please comment on this hypothesis?

Sampson: I may not have a heavily colonized wall, but I have *E. coli* that produce endotoxin. I'm quite impressed by that. I don't think I have endotoxin-producing

Petrunov: Yes, but
which is a normal t

Sampson: Well, you are different than bacteria in there. You heat-killed *E. coli* and

Akdis: It is a very...
whether you have s...
is IgA, IgG1 or IgG

Sampson: Yes. I did not see any IgG1. We are just done that in the past. The mice treated with

de Weck: Are the

Sampson: Well, the

de Weck: Do you think it's resorbed?

Sampson: Actually, the *E. coli*. So we're going to try to track

Lehrer: You change desensitization. I want to go to native molecular response. The second molecules to sensi-

Sampson: The relationship with cholera changed the delivery system. The last slide shows that it is a question of the elements that we are going to use. *E. coli* per rectal and they can become ser-