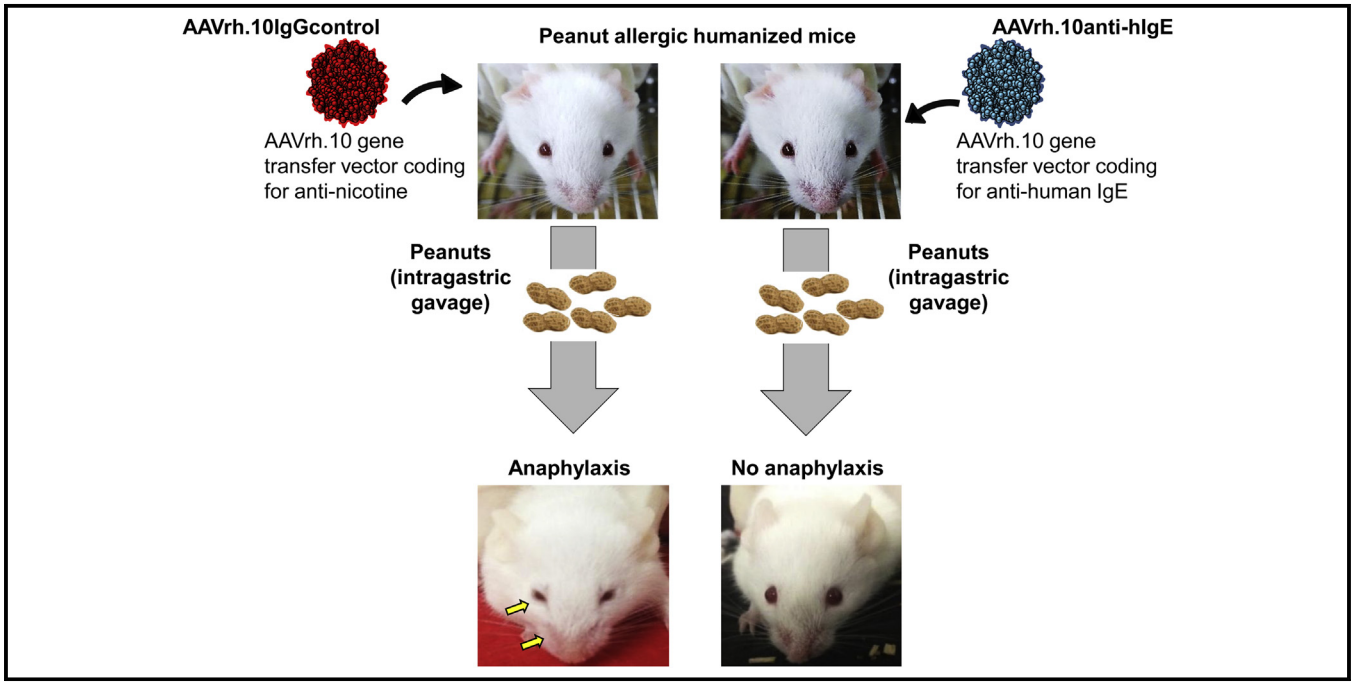
Anti-hIgE gene therapy of peanut-induced anaphylaxis in a humanized murine model of peanut allergy

Odelya E. Pagovich, MD,[a]* Bo Wang, MD,[a,b]* Maria J. Chiuchiolo, PhD,[a] Stephen M. Kaminsky, PhD,[a] Dolan Sondhi, PhD,[a] Clarisse L. Jose, BS,[a] Christina C. Price, MD,[c] Sarah F. Brooks, BSc,[a] Jason G. Mezey, PhD,[a,d] and Ronald G. Crystal, MD[a]* *New York and Ithaca, NY, Sichuan, China, and New Haven, Conn*

GRAPHICAL ABSTRACT



Background: Peanuts are the most common food to provoke fatal or near-fatal anaphylactic reactions. Treatment with an anti-hIgE mAb is efficacious but requires frequent parenteral administration.

Objective: Based on the knowledge that peanut allergy is mediated by peanut-specific IgE, we hypothesized that a single administration of an adeno-associated virus (AAV) gene transfer vector encoding for anti-hIgE would protect against repeated peanut exposure in the host with peanut allergy.

Methods: We developed a novel humanized murine model of peanut allergy that recapitulates the human anaphylactic response to peanuts in NOD-*scid* IL2Rgamma^{null} mice transferred with blood mononuclear cells from donors with peanut allergy and then sensitized with peanut extract. As therapy, we constructed an adeno-associated rh.10 serotype vector coding for a full-length, high-affinity, anti-hIgE antibody derived from the Fab fragment of the anti-hIgE mAb omalizumab (AAVrh.10anti-hIgE). In the reconstituted mice peanut-specific IgE was induced by peanut sensitization and hypersensitivity, and reactions were provoked by feeding peanuts to mice with symptoms similar to those of human subjects with peanut allergy.

Results: A single administration of AAVrh.10anti-hIgE vector expressed persistent levels of anti-hIgE. The anti-hIgE vector,

From [a]the Department of Genetic Medicine, Weill Cornell Medical College, New York; [b]the Department of Respiratory Medicine, West China Hospital, Sichuan University; [c]the Departments of Internal Medicine and Pediatrics, Yale University School of Medicine, New Haven; and [d]the Department of Biological Statistics and Computational Biology, Cornell University, Ithaca.

*These authors contributed equally to this work.

Supported by the Parker B. Francis Fellowship Program and the Department of Genetic Medicine, Weill Cornell Medical College.

Disclosure of potential conflict of interest: O. E. Pagovich is a listed investor on a patent application subsequent to the completion of this work. M. J. Chiuchiolo is a listed investor on a patent application subsequent to the completion of this work. R. G. Crystal serves as a consultant and holds stock options for Adverum Biotechnologies and is a listed investor on a patent application subsequent to the completion of this work. The rest of the authors declare that they have no relevant conflicts of interest.

Received for publication December 10, 2015; revised March 9, 2016; accepted for publication March 28, 2016.

Corresponding author: Ronald G. Crystal, MD, Department of Genetic Medicine, Weill Cornell Medical College, 1300 York Ave, Box 164, New York, NY 10065. E-mail: geneticmedicine@med.cornell.edu.

0091-6749/$36.00

http://dx.doi.org/10.1016/j.jaci.2016.03.053

administered either before sensitization or after peanut sensitization and manifestation of the peanut-induced phenotype, blocked IgE-mediated alterations in peanut-induced histamine release, anaphylaxis scores, locomotor activity, and free IgE levels and protected animals from death caused by anaphylaxis.
Conclusion: If this degree of persistent efficacy translates to human subjects, AAVrh.10anti-hIgE could be an effective 1-time preventative therapy for peanut allergy and possibly other severe, IgE-mediated allergies. (J Allergy Clin Immunol 2016;■■■:■■■-■■■.)

Key words: *Peanut allergy, food allergy, omalizumab, IgE, gene therapy, mouse model*

Peanuts account for the majority of fatal and near-fatal food-induced, anaphylactic reactions in the United States.[1,2] Peanut-induced anaphylaxis typically manifests as vomiting, diarrhea, abdominal pain, angioedema, bronchospasm, hypotension, loss of consciousness, and sometimes death after allergen ingestion.[3] Laryngeal edema, lower-airway obstruction, and hypotension are the most serious manifestations.[4] Unlike other food allergies, peanut allergy is rarely outgrown,[2,5] and it is essential for affected subjects to strictly avoid peanuts and have quick access to an epinephrine autoinjector at all times.[3] Although strides have been made in food allergy awareness and prevention of peanut sensitization, there is no satisfactory therapy to prevent or reverse this disease.[6-9]

Like other food allergies, peanut allergy is mediated by type I hypersensitivity responses linked to peanut antigen–specific IgE.[10-12] The IgE-allergen complex activates mast cells and basophils, with release of mediators responsible for the clinical anaphylaxis response to peanuts (see Fig E1 in this article's Online Repository at www.jacionline.org).[10,12] Based on this knowledge, there has been considerable interest in treating patients with peanut allergy with therapies that interrupt peanut-specific IgE from eliciting anaphylaxis. One approach is treatment with omalizumab (Xolair, Genentech Inc, South San Francisco, Calif, and Novartis Pharmaceuticals Corporation, East Hanover, NJ), a recombinant DNA-derived humanized $IgG_{1\kappa}$ mAb that binds to human IgE.[13] Omalizumab inhibits binding of IgE to the IgE receptor (FcεRI) on the surfaces of mast cells and basophils, thus limiting the degree of release of mediators of the allergic response from the FcεRI-bearing cells. Once circulating IgE antibodies are bound by anti-hIgE, they cannot bind to specific high-affinity Fc receptors on mast cells and basophils, and the complexes of anti-hIgE bound to IgE are then cleared from the circulation.[13] Studies with humanized recombinant anti-hIgE therapy for food allergy have shown promise, at least in the short term.[2,8,13,14] However, using an anti-hIgE mAb as prophylaxis against peanut-induced anaphylaxis in peanut-sensitive subjects has challenges because the protection provided by a single administration of omalizumab is short, estimated at 2 to 4 weeks, requiring at least monthly parenteral administration of the mAb to maintain persistent effective therapy.[15]

As a strategy to circumvent the requirement for repeated anti-hIgE mAb administration, we hypothesized that a single administration of an adeno-associated virus (AAV) coding for omalizumab would provide long-term expression of anti-hIgE, protecting against peanut-induced allergic reactions on a persistent basis. To evaluate this hypothesis, we created a unique humanized mouse model of human IgE-mediated anaphylaxis in response to peanuts. Murine models (not humanized) of peanut allergy are complicated in that both IgE and IgG_1 elicit allergic reactions in mice, whereas IgG antibodies appear to play a smaller role in human anaphylaxis.[16,17] To circumvent this, we created a humanized IgE anti-peanut–induced anaphylaxis NOD-*scid* IL2Rgamma^{null} (NSG) murine model using immunodeficient mice reconstituted with peanut-allergic human blood mononuclear cells. In these mice total and peanut-specific IgE levels are present after engraftment of mononuclear cells from patients with peanut allergy, and peanut-specific anaphylaxis is induced by the release of mediators after challenge with peanut extract. When treated with a single administration of a serotype rh.10 AAV vector coding for omalizumab, there is persistent prevention of peanut-induced severe allergy, either when administered as prophylaxis before peanut sensitization or as therapy after the mice exhibit the peanut-induced anaphylaxis–related symptomology.

Abbreviations used
AAV: Adeno-associated virus
GVHD: Graft-versus-host disease
hIgE: Human IgE
NSG: NOD-*scid* IL2Rgamma^{null}
PCA: Passive cutaneous anaphylaxis

METHODS

Humanized murine model

NSG mice (6-8 weeks old) were housed in microisolator cages and maintained on peanut-free chow, according to standard guidelines. All food and water was autoclaved. Mice were bred as pairs (1 female with 1 male) or trios (2 females with 1 male); only female mice were used in the allergy experiments because female mice have been found to be more susceptible to anaphylaxis than male mice.[18]

All experiments using human cells and sera were approved by the institutional review boards at Weill Cornell Medical College and Yale New Haven Hospital, and written informed consent was obtained from each subject enrolled in the study. Heparinized blood was obtained from donors with allergy to peanut or nonallergic healthy control subjects (see Table E1 in this article's Online Repository at www.jacionline.org). Specific sensitization was documented by detection of allergen-specific IgE in the sera of donors (ImmunoCAP specific IgE blood test; Phadia AB, Uppsala, Sweden). Blood mononuclear cells were isolated from heparinized blood by using Ficoll-Paque density centrifugation (Ficoll-Paque Plus; Sigma-Aldrich, St Louis, Mo). The absence of detectable human IgG in mouse sera was assessed by means of ELISA before reconstitution with human cells.

Cells isolated by using the Ficoll-Paque method were administered to 6- to 8-week-old NSG mice. Each animal received intraperitoneally 3×10^7 blood mononuclear cells in RPMI (Sigma-Aldrich) mixed with 100 μg of crude peanut extract in a total volume of 200 μL of 0.9% sodium chloride split in 2 separate injection sites (100 μL each).

Protein extracts from roasted unsalted peanuts (*Arachis hypogaea*; Hampton Farms, Severn, NC) were made on the same day of administration to mice by mixing 25 g of ground peanut with 250 mL of 20 mmol/L Tris buffer (pH 7.2). After 2 hours at 23°C, the aqueous fraction was collected and subsequently centrifuged to remove residual traces of fat and insoluble particles.[19] Protein concentrations were determined by using Bradford analysis with BSA as a standard. The presence of expected peanut allergens in the peanut extract preparation was confirmed by using SDS-PAGE (see Fig E2 in this article's Online Repository at www.jacionline.org). Mice were sensitized once weekly at weeks 0 to 4 with 100 μg of crude peanut extract through intraperitoneal injections. Mice were then challenged at weeks