The Consortium for Food Allergy Research (CoFAR): The first generation

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The Consortium for Food Allergy Research (CoFAR) was established by the National Institute of Allergy and Infectious Diseases in 2005 as a collaborative research program bringing together centers focused on the study of food allergy. CoFAR was charged with developing studies to better understand the pathogenesis and natural history of food allergy, as well as potential approaches to the treatment of food allergy. In its first iteration an observational study of infants with milk and egg allergy was established, and studies of oral immunotherapy for egg allergy and sublingual immunotherapy for peanut allergy were initiated, as was a phase 1 study of a recombinant peanut protein vaccine. CoFAR was renewed in 2010 for an additional 5-year period during which the initial observational study was continued, a study of eosinophilic esophagitis was initiated, and new therapeutic trials were established to study epicutaneous immunotherapy for peanut allergy and to compare the safety and efficacy of egg oral immunotherapy to the ingestion of baked egg for the treatment of egg allergy. The results of these efforts will be reviewed in this rostrum, with a brief look to the future of CoFAR. (J Allergy Clin Immunol 2019;143:486-93.)

Key words: *Food allergy, peanut allergy, egg allergy, immunotherapy, oral immunotherapy, sublingual immunotherapy, epicutaneous immunotherapy*

Abbreviations used

AD:	Atopic dermatitis
CoFAR:	Consortium for Food Allergy Research
EoE:	Eosinophilic esophagitis
EPIT:	Epicutaneous immunotherapy
NIAID:	National Institute of Allergy and Infectious Diseases
OFC:	Oral food challenge
OIT:	Oral immunotherapy
RFA:	Request for application
SCD:	Successfully consumed dose
SLIT:	Sublingual immunotherapy
SPT:	Skin prick test
SU:	Sustained unresponsiveness
Treg:	Regulatory T
VP:	Viaskin Peanut

On direction from Congress, the National Institute of Allergy and Infectious Diseases (NIAID) convened an Expert Panel on Food Allergy Research in June 2003 to evaluate the current state of IgE-mediated food allergy and to make recommendations for targeted basic science and clinical research. The expert panel recommended new initiatives to eliminate critical gaps in the prevailing understanding of gastrointestinal physiology, immunology, and the mechanism of oral tolerance; the pathophysiology of food allergy and food allergy-associated anaphylaxis; and the molecular characteristics of food allergens. The panel also recommended targeted research to define the natural history of childhood and adult-onset food allergy, including the importance of

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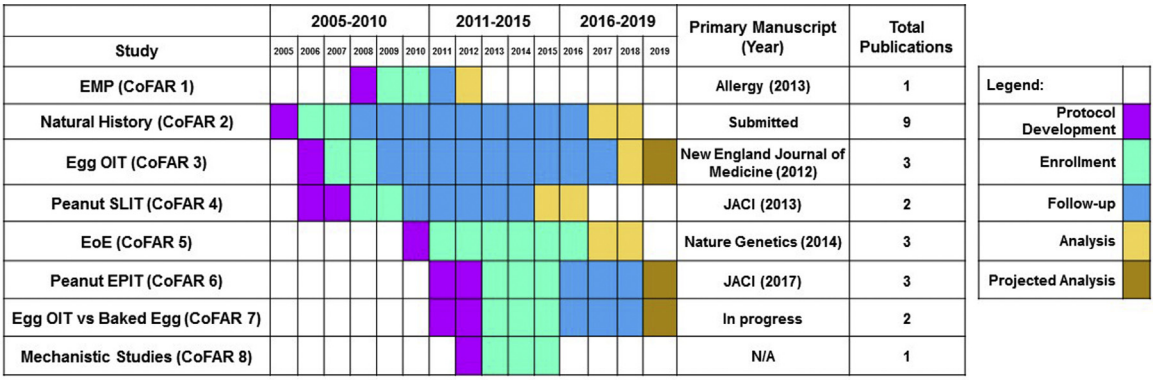

FIG 1. CoFAR timeline. *EMP*, EMP-123; *JACI, Journal of Allergy and Clinical Immunology.*

understanding spontaneous re-establishment of tolerance after development of allergy, and to develop new immune-based therapies to prevent and treat food allergy.

In August 2004, the NIAID announced a request for application (RFA) "to establish a Food Allergy Research Consortium, a collaborative research program designed to develop new approaches to treat and prevent food allergy," as well as applications to establish a statistical and clinical coordinating center to support the clinical research projects initiated by the Consortium. The goals specified in the original RFA to be addressed by the program included the following: (1) develop immune intervention strategies to prevent and treat food allergy; (2) identify the mechanisms of development, loss, and re-emergence of oral tolerance; (3) determine the molecular and functional characteristics of food allergens; and (4) determine the role of the gastrointestinal tract in the development and loss of oral tolerance. These were very ambitious goals, and many of the knowledge gaps to be addressed remain today, but since this original call to arms 14 years ago, this program has resulted in many major advances and influenced the rigor and standards of clinical research in food allergy worldwide.

After a competitive review process, the NIAID awarded the initial grant in July 2005 to the Icahn School of Medicine in New York and its academic partners: Duke University Medical Center (Durham, NC), the University of Arkansas Children's Hospital Research Institute (Little Rock, Ark), Johns Hopkins University School of Medicine (Baltimore, Md), and National Jewish Children's Medical and Research Center (Denver, Colo). The EMMES Corporation (Rockville, Md) was awarded a contract to serve as the statistical and clinical coordinating center. In 2010, the Consortium for Food Allergy Research (CoFAR) was renewed for an additional 5-year period with cofunding by the NIAID and the National Institute of Diabetes and Digestive and Kidney Diseases and with the addition of Cincinnati Children's Hospital Medical Center to incorporate the investigation of eosinophilic esophagitis (EoE) in addition to an ongoing observational study and new therapeutic trials.

Over its first 10 years, CoFAR investigated a variety of immunotherapeutic approaches, including oral immunotherapy (OIT), sublingual immunotherapy (SLIT), and epicutaneous immunotherapy (EPIT), as well as an engineered recombinant peanut protein rectal vaccine (Fig 1). These clinical trials, along with the observational study, have resulted in 42 abstracts presented at various international meetings and 30 articles to date, about one half of which were published in the *Journal of Allergy and Clinical Immunology* but also in other high-impact journals, such as the *New England Journal of Medicine*, *Nature Genetics*, and *Nature Communications*, among others. In this rostrum we provide a brief overview of the accomplishments of the NIAID-sponsored CoFAR, as well as a glimpse into the future with the current CoFAR, which was refunded in 2017.

OBSERVATIONAL INSIGHTS FROM CoFAR STUDIES

Natural history of egg, milk, and peanut allergy

CoFAR recruited a cohort of 512 infants aged 3 to 15 months with likely egg or milk allergy and no known peanut allergy to investigate immunologic, genetic, and environmental factors that determine the natural course of egg and milk allergy and the development of peanut allergy (the study is referred to as the CoFAR 2 Observational study).[1] Entry criteria required moderate-to-severe atopic dermatitis and a positive skin prick test (SPT) response to egg or milk, a clinical history of egg or milk allergy and a confirmatory positive SPT response, or both. Infants with known or likely peanut allergy, including those having had a reaction or having a prestudy peanut-specific IgE antibody level of greater than 5 kU_A/L, were excluded from enrollment to increase the power of the study for identifying factors associated with development of peanut allergy (there were 104 infants excluded from enrollment based on these criteria). Participants were evaluated every 6 months for 1 year and then yearly, with telephone calls between visits to review any allergic reactions. Tests included SPTs, phlebotomy, physical examinations, dietary and medical histories, and oral food challenges (OFCs). Because this was an observational study, there were no protocol-mandated periodic OFCs. Determination of allergy status to egg, milk, and peanut was based on criteria that included reaction/ingestion history, skin and serum test results, and OFCs, as clinically indicated. Enrollment began in July 2006 and ended in March 2008 at a time when the American Academy of Pediatrics guidelines for the prevention of atopic disease were suggesting avoidance of allergenic foods in highly atopic infants,[2] which was the general approach at the time of enrollment but not as the study progressed because those recommendations were rescinded in 2008.[3] Additional inclusion/exclusion criteria are described elsewhere.[1]

The cohort consisted of 512 infants, 60.2% of them enrolled with clinical reactions to egg or milk and the remainder having moderate-to-severe atopic dermatitis and a positive skin test

response to milk or egg. The median age at enrollment was 9 months. Some degree of breast-feeding was common (85.7%). Rates of sensitization were high, with 77.7% sensitized to milk, 88.7% to egg, and 68.8% to peanut. Although exclusions were in place to reduce the number of infants enrolled with likely peanut allergy, 26.6% of infants were found to have a serum peanut-specific IgE level of greater than 5 kU_A/L after enrollment. The characteristics of the cohort could be considered typical for infants referred to allergists for a food allergy evaluation and also similar to those of infants at high risk of peanut allergy, as evaluated in the Learning Early About Peanut trial.[4]

Enrollment features of subjects with milk and egg allergies were analyzed against outcomes of persistent or resolved allergy to determine early markers of prognosis of these allergies. For milk, 53% (154/293) experienced allergy resolution at a median age of 63 months.[5] For egg, 49% (105/213) resolved the allergy at a median of 72 months.[6] Numerous baseline clinical and laboratory markers were analyzed for their ability to predict outcomes. For milk, baseline characteristics that were most predictive of resolution included milk-specific IgE level, milk SPT wheal size, and atopic dermatitis severity.[5] For egg, factors that were most predictive of resolution included initial reaction characteristics (isolated urticaria/angioedema vs other presentations), baseline egg-specific IgE level, egg SPT wheal size, atopic dermatitis severity, and IgG_4 and IL-4 response, but in a multivariate analysis the egg-specific IgE level and initial reaction characteristics best informed the outcome.[6] Calculators were devised that graph predicted outcomes based on the parameters identified in multivariate analysis and are available at https://web.emmes.com/study/cofar/index.htm.

Regarding the development of peanut allergy, the results thus far have been presented in a meeting abstract.[7] Briefly, over the course of the study with follow-up to a median age of 8 years, 40% of the cohort had a diagnosis of peanut allergy. Important enrollment factors associated with peanut allergy included peanut sensitization status and breast-feeding (which was a protective factor). A comprehensive evaluation of factors associated with risk or protection from peanut allergy in the cohort is underway. However, the risks for presenting on enrollment with likely peanut allergy, which was defined as a peanut IgE level of greater than 5 kU_A/L, were also evaluated.

In an initial study that did not include analysis of environmental exposure to peanut in the home, frequent maternal ingestion of peanut during pregnancy (ie, twice per week or more) was a significant predictor (odds ratio, 2.9), along with male sex, nonwhite race, and increased egg and milk IgE levels.[8] A subsequent study evaluated the amount of peanut in household dust of the infants and incorporated this factor in the analysis.[9] There was a dose-response relationship between the amount of environmental peanut and the infant being enrolled with a peanut IgE level of greater than 5 kU_A/L, and the relationship was augmented by increasing severity of atopic dermatitis. These findings, at a time when early peanut ingestion was not being encouraged, provide additional evidence of the role of skin exposure, with absent oral exposure being a risk factor for peanut allergy.

Stool samples were collected from the participants at enrollment and provided the opportunity to explore the association of gut microbiome composition, as profiled by using 16s rRNA sequencing, with outcomes. Regarding milk allergy, resolution was associated with enrichment of *Clostridia* and *Firmicutes* species, suggesting potential targets for probiotic candidates for treatment.[10] Regarding egg allergy, no associations could be found for predicting resolution, but unique genera of bacteria were abundant in the children with egg allergy.[11] Studies on peanut allergy outcomes are pending.

Insights regarding food allergen exposure and reactions during longitudinal evaluation of the cohort have provided additional clinical lessons. Clinical reactions were recorded in real time because participants were instructed to notify the centers of a reaction, and reactions were also captured at scheduled telephone calls or visits. Despite being evaluated in 5 dedicated food allergy referral centers and receiving standard instructions about avoidance and treatment, the rate of annualized reactions was 0.81 over a median follow-up of 36 months from enrollment.[12]

Additionally, the studies uncovered a number of features of the reactions that are of interest for counseling families, such as lack of vigilance, purposeful trials of avoided foods, and underuse of epinephrine. The high rate of reactions and underuse of epinephrine raise the issue of how to best inform families on food allergy care. Using supplemental funding to CoFAR, studies were conducted,[13-16] leading to a program consisting of a number of educational materials that were validated to reduce accidental reactions and are available online (https://web.emmes.com/study/cofar/index.htm). The unintended allergic reactions experienced by the participants, along with OFCs performed as needed, also provided data to address the question of whether accidental or purposeful exposure leading to a reaction causes a boost in IgE levels to the causal food, a concern that might inhibit parents from pursuing OFCs. In evaluating 20 to 27 OFCs with reactions for each food and more than 446 accidental reactions, the data support the conclusion that these exposures were not associated with significant increases in sensitization to milk, egg, or peanut.[17] Table I[13,17,18] provides practical lessons from these studies that could be used for educating families about food allergy management.

CoFAR Eosinophilic Esophagitis Registry

CoFAR also sought to gain insights into EoE by establishing a registry enrolling children and adult subjects with this disease.[18] The registry includes 705 subjects, with age at enrollment ranging from 0.9 to 56.2 years (median, 11.2 years). Failure to thrive was common (21%), and gastrointestinal eosinophilia was noted in about 10% of the cohort. Some of the clinical findings included the following: significant time lags between symptoms and diagnosis that were greater for older patients (eg, median 4-year time lag for adults and 1 year for those <11 years of age), less delay in diagnosis for those with atopic dermatitis or food allergy, and high rates of allergic disease (91%), infectious and immune disorders (44%), and neurodevelopmental disorders (30%). The rate of EoE in parents was 3%, and that in siblings was 4.5%.

One of the primary goals of the registry was to inform the genetic basis of EoE, and the registry was developed in collaboration with the Cincinnati Children's Hospital Medical Center to perform genetic analysis on samples collected from the subjects. Reports from this collaboration thus far have identified a number of putative susceptibility loci for EoE and elucidated a likely role of *CAPN14* (encoding calpain 14, a calcium-activated cysteine protease) in the tissue-specificity and allergic disease–linked aspects of the disorder[19] and risk loci at 16p13 that links EoE with 10 other immune-associated diseases.[20] These

TABLE I. Findings from CoFAR observational studies (CoFAR 2 and CoFAR 5) with management pearls for the clinician

Clinical finding/observation	Implication/clinical pearl/counseling point
Accidental or OFC exposure with reaction to egg, milk, or peanut did not boost IgE sensitization.[17]	OFCs should not be deferred for concerns of increasing sensitization.
Despite receiving standard instructions about avoidance and treatment, the rate of annualized reactions was 0.81 over a median follow-up of 36 months from enrollment, and rescue epinephrine was underused.[13]	Accidental (and purposeful) ingestion of food allergens is not uncommon, and epinephrine rescue is underused, emphasizing the need for repeated education of patients and caregivers.
About 2 of 3 accidental ingestions resulting in reactions were caused by lack of vigilance.[13]	Families should be educates about supervision, reading labels, discussing allergy with restaurant staff, cross-contacts, and hidden ingredients.
About half of reactions occurred when not under parental supervision.[13]	Education should be extended to relatives, friends, and caregivers.
Purposeful trying of avoided foods accounted for 11% of reactions.[13]	Health care personnel should ensure that families are comfortable with the diagnosis and know not to attempt home trials.
Epinephrine was not given for 30% of severe reactions.[13]	Families should be educated regarding the safety and utility of prompt administration of epinephrine.
Delayed EoE diagnosis is common.[18]	A high index of suspicion for EoE should be maintained.
Comorbidity in patients with EoE is common.[18]	The possibility of coincident eosinophilic gastritis and immune and neurodevelopmental disorders is to be considered.

various findings provide clinical insights and a means to reach better diagnostic and therapeutic outcomes.

THERAPEUTIC INSIGHTS FROM CoFAR STUDIES

Intervention trials

CoFAR has conducted a series of interventional studies on the treatment of food allergy, including food antigens administered through the SLIT, OIT, and EPIT routes, and a novel recombinant peanut protein, all in an effort to lead the way forward toward determining optimal treatments for food allergy. These studies will be reviewed here, focusing primarily on clinical outcomes (Table II).[21-26]

In CoFAR 1, the first CoFAR treatment protocol, we studied the safety and immunologic effects of a vaccine containing recombinant modified peanut proteins. The rationale for the development of this vaccine was the potential to induce tolerance with a lower risk of adverse reactions through modification of the peanut proteins. This product, referred to as EMP-123, was a rectally administered suspension of recombinant Ara h 1, Ara h 2 and Ara h 3 modified by amino acid substitutions at major IgE-binding epitopes and encapsulated in heat/phenol-killed *Escherichia coli*. In 2013, we published results of this phase 1 study of EMP-123, in which 5 healthy volunteers and then 10 adults with peanut allergy were treated.[21] Unfortunately, adverse reactions were common among the volunteers with peanut allergy, including 5 in whom adverse reactions prevented completion of dosing and 2 with anaphylactic reactions. These results suggested that any future studies using this product would require changes to the dosing scheme, route of delivery, or both.

In CoFAR 3 we conducted a trial of OIT for the treatment of egg allergy, the first double-blind, placebo-controlled study of OIT for any food conducted at more than 2 sites.[22] Fifty-five children from 5 to 11 years of age were treated with OIT (40 children) or placebo (15 children). A maintenance dose of 2 g of egg white powder was used, which is approximately 1.6 g of egg white protein. No baseline OFCs were performed, but OFCs were completed after 10 and 22 months of therapy. Children who successfully passed a 10-g challenge at 22 months discontinued the OIT and avoided all egg consumption for 6 to 8 weeks, after which they underwent another OFC to assess for sustained unresponsiveness (SU). After 10 months of therapy, none of the children who received placebo and 55% of those who received active OIT passed a 5-g OFC and were considered desensitized. After 22 months, 30 (75%) of the children in the OIT group were desensitized to 10 g; however, only 11 (28%) of the 40 children passed the OFC after the period of avoidance and were considered to have SU. At follow-up at 30 and 36 months, all of the children who had achieved SU were consuming egg. We concluded that egg OIT can desensitize a high proportion of children with egg allergy but only induce SU in a subset.

A follow-up of the CoFAR 3 study was also conducted to assess longer-term results, including both those who had moved to dietary intake after demonstrating SU and those without SU who remained on egg OIT through months 36, 48, or both.[23] We found that of the 40 subjects originally treated with egg OIT, 18 (45%) and 20 (50.0%) demonstrated SU by years 3 and 4, respectively. For those subjects still dosing during years 3 and 4, mild symptoms were still reported in 12 (54.5%) of 22 subjects. At the time of this long-term follow-up, more subjects receiving egg OIT were consuming egg versus placebo, with 90% of those achieving SU consuming all forms of egg.

CoFAR 4 focused on SLIT for peanut allergy.[24] This was a randomized, double-blind, placebo-controlled multicenter trial of peanut SLIT in adolescents and adults. After a baseline OFC of up to 1 g of peanut protein, 40 subjects aged 12 to 37 years were randomized 1:1 to daily placebo or peanut SLIT (maintenance dose, 1.4 mg). A 5-g OFC was performed after 44 weeks, followed by unblinding. Placebo-treated subjects then crossed over to higher-dose peanut SLIT (3.7 mg), followed by a week 44 OFC. Subjects who successfully consumed 5 g in the week 44 OFC or at least 10-fold more peanut compared with the baseline OFC were considered responders.

We found that after 44 weeks of SLIT, 14 (70%) of 20 subjects receiving peanut SLIT were responders compared with 3 (15%) of 20 receiving placebo ($P < .001$). The median successfully consumed dose (SCD) at week 44 was significantly higher than the baseline OFC for peanut SLIT–treated subjects (371 vs 21 mg) but not for placebo-treated subjects (146 vs 71 mg). However, the median SCD was not significantly different between the active and placebo groups. With more than 10,000 peanut doses through the week 44 OFCs, 63.1% of doses were symptom free, and when oral-pharyngeal symptoms were excluded, 95.2% were symptom free. We concluded from this

TABLE II. Findings from CoFAR intervention trials

	Trial design	Major findings
CoFAR 1	Phase 1 study of a vaccine containing recombinant modified peanut proteins[21]	Adverse reactions were too common to pursue additional studies.
CoFAR 3	DBPC trial of egg OIT[22]	Egg OIT can desensitize a high proportion of children with egg allergy but only induce SU in a subset.
CoFAR 3 FU	FU of egg OIT through 48 months[23]	Of the total, 50.0% demonstrated SU by years 4; more subjects receiving egg OIT were consuming egg versus placebo, with 90% of those achieving SU consuming all forms of egg.
CoFAR 4	DBPC trial of peanut SLIT[24]	Peanut SLIT safely induced a modest level of desensitization in a majority of subjects compared with placebo.
CoFAR 4 FU	FU of peanut SLIT through 36 months[25]	Peanut SLIT safely induced a modest level of desensitization, but SU was uncommon; 50% discontinued treatment.
CoFAR 6	DBPC trial of peanut EPIT[26]	Peanut EPIT was safe and associated with a modest treatment response after 52 weeks, with the highest responses among younger children.
CoFAR 7	Comparison of egg OIT with baked egg	Results pending

DBPC, Double-blind, placebo-controlled; *FU*, follow-up.

study that peanut SLIT safely induced a modest level of desensitization in a majority of subjects compared with placebo.

Treatment was continued for as long as 3 years in CoFAR 4, and we subsequently published a long-term follow-up study of these subjects, assessing response rates at 2 and 3 years, as well as SU, in those who were fully desensitized in the 5-g OFCs.[25] Data were somewhat limited by the fact that more than 50% discontinued therapy. By study's end, 4 (10.8%) of 37 SLIT-treated participants were fully desensitized, and all 4 achieved SU. Approximately 98% of the more than 18,000 doses administered were tolerated without adverse reactions beyond the oropharynx, with no severe symptoms or use of epinephrine. We again concluded that peanut SLIT induced a modest level of desensitization and had an excellent long-term safety profile. The low rate of SU was probably not surprising, but we were struck by the high discontinuation rate, especially in view of the excellent safety profile.

CoFAR 6 again focused on peanut but this time using EPIT with Viaskin Peanut (VP).[26] This was a multicenter, double-blind, randomized, placebo-controlled study that included 74 participants aged 4 to 25 years. Subjects were randomized to treatment with placebo, 100 mg of VP (VP100), or 250 mg of VP (VP250; DBV Technologies, Montrouge, France). The primary outcome was treatment success after 52 weeks, which was defined as passing a 5044-mg OFC or achieving a 10-fold or greater increase in SCD from baseline to week 52. At week 52, treatment success was achieved in 3 (12%) placebo-treated participants, 11 (46%) VP100 participants, and 12 (48%) VP250 participants, with significant differences between the placebo group and both active groups but no difference between the VP100 and VP250 groups. Median changes in SCDs were 0, 43, and 130 mg of protein in the placebo, VP100, and VP250 groups, respectively. Treatment success was greater among children aged 4 to 11 years compared with that among subjects older than 11 years. Overall, 14.4% of placebo doses and 79.8% of VP100 and VP250 doses resulted in adverse events, predominantly local patch-site reactions. We concluded that peanut EPIT administration was safe and associated with a modest treatment response after 52 weeks, with the highest responses among younger children. The study is ongoing, with results after 130 weeks of treatment pending at this time.

In CoFAR 7 we returned to the study of egg allergy with a protocol comparing egg OIT to ingestion of extensively heated egg. This protocol was recently completed, and data are under analysis at this time.

MECHANISTIC INSIGHTS FROM CoFAR STUDIES

For more information on mechanistic insights from CoFAR studies, see Table III.[1,6,7,25,27-29]

Food-specific immunoglobulins

The CoFAR observational and intervention studies have provided opportunities to better understand the relationship of food-specific IgE to clinical reactivity. The magnitude of the food-specific IgE response clearly does matter for clinical outcome. In the observational cohort the greater the level of milk-specific[5] or egg-specific[6] IgE at baseline enrollment (3–15 months of age), the lower the likelihood of allergy resolution by 72 months of age. Food-specific IgE is also a predictor of the clinical outcome of OIT. Levels of egg- and ovalbumin-specific IgE at baseline are significantly and negatively associated with development of SU after egg OIT.[30] Egg-specific IgE levels are also negatively associated with baked egg tolerance, as demonstrated in the baseline challenges in CoFAR 7.[27]

These data tell us that the magnitude of the food-specific IgE response is fundamental to the prognosis of food allergy, yet the food-specific IgE level remains a flawed biomarker because of the overlap in levels between clinical groups. One approach to improve the precision of this measure is to incorporate epitope specificity. In preliminary data from CoFAR, measurement of epitope-specific IgE to Ara h 1, 2, and 3 and application of machine learning algorithms could predict clinical peanut allergy in the natural history cohort with greater accuracy than peanut- or Ara h 2–specific IgE.[31]

The most consistent effect of allergen immunotherapy is the induction of allergen-specific IgG_4. This is observed with OIT, SLIT, and EPIT.[22,24,26] The magnitude of the food-specific IgG_4 response is generally greatest for OIT, which has the greatest exposure dose, as well as treatment response. The magnitude of the egg-specific IgG_4 response early in OIT was found to be associated with development of SU,[22] although others have not found IgG_4 levels to be predictive of SU.[32,33] It remains unclear whether IgG_4 is a mechanism of protection or a measure of

TABLE III. Summary of findings from CoFAR mechanistic studies

Finding	Implication/speculation
Levels of milk- and egg-specific IgE are predictive of natural resolution.[6,8]	Understanding the regulation of allergen-specific IgE production is critical to understanding the natural resolution of food allergy.
Basophil activation test results were predictive of changes in reaction threshold after immunotherapy,[26] and results were significantly different between baked egg–reactive and tolerant subjects.[27]	Basophil activation tests might prove useful for predicting clinically important phenotypic differences within food allergies.
Numbers of T_H2 cells were found to be increased in patients with milk, egg, and peanut allergy.[1,27,28] Cytokines derived from highly differentiated T_H2 cells were more resistant to Treg cell suppression.[28]	Differentiation status of T_H2 cells might be critical in determining the susceptibility of the immune system to generate tolerance after immunotherapy.
Treg cells and expression of regulatory genes were not found to be different between patients with food allergy and control subjects.[1,27,28]	Deletion of T_H2 cells rather than counterregulation by Treg cells might be more effective in the treatment of food allergy.
Transcriptional profiling of whole blood revealed a gene signature and key driver genes associated with anaphylaxis to peanut.[29]	Identification of driver genes, such as leukotriene B_4 and *IL1R2*, and cell types, including neutrophils and macrophages, associated with peanut-induced anaphylaxis reveal novel pathways that need to be investigated as a source of new therapeutic targets.

exposure. IgG_4 can function as a blocking antibody, can suppress allergic effector cell activation through FcγRIIb,[34] and can facilitate the development of immune tolerance,[35] likely by changing the phenotype of the antigen-presenting cell after IgG-facilitated uptake and presentation. IgG_4 levels were not predictive of clinical outcome in the natural history cohort, suggesting that they might not play a role in natural resolution of food allergy. The mucosal routes of allergen immunotherapy also generate a food-specific IgA response[30,36] that can contribute to clinical tolerance through immune exclusion.

Basophil activation tests

Basophil activation tests have been performed as biomarkers of response to OIT, SLIT, and EPIT.[22,24,26] Egg OIT was associated with a significant reduction in basophil activation,[22] and basophil activation discriminated between those who passed versus failed their OFCs. Basophil activation tests from SLIT- or EPIT-treated subjects also revealed a shift in basophil activation, such that significant reductions of activation are observed at lower doses of allergen.[24,26] Basophil activation tests also revealed modest differences between those with reactivity or tolerance to baked egg, with significantly lower activation in the baked egg–tolerant group at lower concentrations of egg allergen.[27] To date, the basophil activation test has not yet been harnessed for routine clinical use to guide clinical care because of the need to activate basophils within a few hours of obtaining the blood sample but appears to be sensitive to relatively modest changes in clinical reactivity.

Allergen-specific T cells

The allergen-specific T-cell response was monitored by using 2 distinct approaches in the CoFAR studies. The first, which was used in the natural history cohort, used CD25 selection after allergen stimulation to enrich for activated and regulatory T (Treg) cells. This enriched fraction was then lysed for RNA isolation and quantitative RT-PCR for a panel of genes of interest. By using this approach, expression of IL-4 in the enriched fraction after milk or peanut stimulation was found to be predictive of milk and peanut allergy, respectively.[1] No regulatory genes were found to be predictive of food allergy. The advantage of this approach was that it captured both activated and Treg cells and required relatively small blood volumes. Limitations of the approach included the capture of basophils and natural killer cells together with T cells using CD25 selection, which might have contributed to the IL-4 signal.[37]

A second approach used the activation marker CD154 (or CD40 ligand) to detect allergen-responsive T cells after short-term stimulation (6-18 hours) with food allergen extract. This approach captures all potential epitopes that can be derived from the food extract and elicits antigen-specific production of cytokines that can be detected by using intracellular cytokine staining. We used this approach to phenotype the peanut-specific T-cell response in baseline samples from 75 subjects enrolled in the CoFAR 6 peanut EPIT trial.[28] We compared the T-cell response to a peanut-sensitized group that passed the 1-g enrollment challenge to peanut and were therefore excluded from enrollment in CoFAR 6, as well as a group of healthy control subjects. Using this short-term stimulation approach, we found that peanut allergy was associated with a significant peanut-specific T_H2 response (IL-4 and IL-13) and an absence of other cytokines, such as IFN-γ or IL-17. The control groups had an absence of any detectable peanut-responsive T cells, including IFN-γ– or IL-10–producing cells. A subset of IL-4–expressing T cells coexpressed IL-5 and IL-9 and lost expression of CD27, which is indicative of terminal differentiation. Single-cell RNA sequencing analysis of peanut-responsive T cells identified the expression of a cluster of T_H2 genes, including *IL9*, *IL5*, *IL4*, *IL13*, and the IL-25 receptor *IL17RB*, as highly differentially expressed in peanut-responsive T cells. The phenotype of these cells is consistent with that of T_H2A cells described by Wambre et al,[38] with the exception of the marker chemoattractant receptor–homologous molecule expressed on T_H2 lymphocytes, which we did not find to be expressed.

We observed that CD154 was upregulated on $CD25^+CD127^{low}Foxp3^+$ Treg cells after peanut stimulation, with a delayed kinetics compared with $CD4^+$ effector cells (18 vs 6 hours).[28] This CD154 response of Treg cells was observed in patients with peanut allergy but surprisingly not in peanut-sensitized or healthy control subjects. Neutralization experiments demonstrated that this Treg cell response, but not the early effector response, was dependent on IL-2, indicating that Treg cells can upregulate CD154 as a response to effector cell activation.

The egg-specific T-cell response was also quantified at baseline in children with egg allergy enrolled in CoFAR 7. We had the opportunity to profile the T-cell response from 129 children with egg allergy, including 81 with clinical reactions to baked egg and 48 who tolerated baked forms of egg.[27] The phenotype of the egg-specific T-cell response was similar to that to peanut, including a $CD4^+$ T-cell response dominated by T_H2 cytokines and a Treg cell response with delayed kinetics. Egg-responsive T_H2 effector and Treg cells expressed CCR4 and CCR6. When comparing those who reacted to or tolerated baked egg, we found no significant differences in the effector T or Treg cell response. However, we did observe a subset of high T_H2 responders only in the baked egg–reactive group. We speculate that this high T_H2 response will have implications for disease persistence.

Transcriptomics of food allergy

In addition to hypothesis-driven studies on T-cell phenotypes in patients with food allergy, we have also incorporated data-driven approaches to study the immune response to allergen exposure. We examined gene expression in PBMCs from patients with egg allergy and atopic control subjects after 24 hours of stimulation with egg white extract.[39] Gene expression was assessed in the bulk PBMC population by using microarray analyses to study the immune response of all mononuclear cells to allergen. Genes that were differentially expressed in the group with egg allergy included T_H2 cytokines and chemokines, such as IL-9 and CCL17. We identified coexpressed gene modules using weighted gene coexpression network analysis, resulting in 5 modules that were significantly enriched for the egg allergy gene signature. Enrichment analysis using an immune annotation resource that we curated identified pathways expected to be associated with egg allergy (T_H2 and Treg cells) but also identified strong associations with myeloid cells and pathways activated by Toll-like receptor 4 signaling. We also compared gene expression induced by egg in baked egg–reactive and tolerant individuals. Differentially induced genes between these 2 phenotypes of egg allergy included genes associated with interferon signaling and virally infected dendritic cells. This hypothesis-generating approach suggested a key role for innate cells, particularly dendritic cells, in the food-induced allergic immune response, which will be addressed in future studies.

Mechanistic studies performed within CoFAR have also examined the molecular basis of peanut-induced anaphylaxis through transcriptomics of whole blood. Watson et al[29] obtained blood samples from children undergoing peanut and placebo food challenges. Blood samples were obtained at baseline and 2 and 4 hours after the start of food challenge, and gene expression was analyzed in the discovery and replication cohorts. Several genes were identified as upregulated during peanut-associated anaphylaxis, including the leukotriene B_4 receptor and *IL1R2*, a decoy receptor that neutralizes IL-1β. Leukocyte deconvolution approaches used changes in gene expression to identify changes in cellular composition associated with peanut-associated anaphylaxis. Expression of genes associated with macrophages and neutrophils was increased, whereas expression of genes associated with naive T cells was decreased. Analysis of gene coexpression by using weighted gene coexpression network analysis identified 13 gene modules, one of which was significantly enriched for genes regulated during peanut-associated anaphylaxis. Gene ontology revealed "acute-phase response" as the pathway most highly associated with this gene module.

Key driver analysis was used to identify genes that are most upstream in the network of genes regulated during peanut anaphylaxis. Six genes, including *LTB4R* and *IL1R2*, were identified as key drivers of the peanut-induced anaphylaxis response. This data-driven discovery approach identified novel cell types, as well as immune processes, activated during peanut-induced anaphylaxis, resulting in several new potential treatment targets for peanut allergy worthy of investigation.

LESSONS LEARNED

Over its first 13 years, CoFAR has added tremendously to our knowledge based on the natural history of food allergy, mechanisms and biomarkers of food allergy and EoE, and potential therapeutic strategies. Among the many lessons learned, a few stand out as this field of food allergy rapidly moves forward.

CoFAR 1 taught us that modification of food antigens, in an effort to reduce reactivity, is not as simple as it once seemed. Although CoFAR 2 was designed to study risk factors for the development of peanut allergy in infants with milk and egg allergy, we learned that a large subset of these infants already had peanut allergy. CoFAR 3 taught us that OIT can effectively desensitize most children with egg allergy, the word "tolerance" needed to be removed from our concept of desensitization and replaced with the more modest term of SU, and even SU is difficult to accomplish. Finally, in CoFAR 4 and CoFAR 6 we learned that safer means of desensitization might be possible but that the efficacy of SLIT and EPIT are far less than that seen with OIT. We also learned lessons from these studies about the choice of end points for food immunotherapy, recognizing that a simple 10-fold increase in challenge threshold might not be an adequate marker of desensitization.

Overall, the scientific rigor of CoFAR's mechanistic studies and clinical trials has helped raise the standards for research in this field, brought increased attention to the science of food allergy worldwide, and helped attract new young investigators and experienced veterans from other scientific areas to the discipline. With the recent renewal of CoFAR and the influx of new talent, the future of food allergy research appears bright and the possibilities for improving the lives of our patients seems close at hand.

CoFAR: THE NEXT GENERATION

CoFAR was refunded in 2017 for an additional 7 years. CoFAR is now funded by 2 separate RFAs: one for a single leadership center and one for multiple clinical research units. The clinical sites that were funded in 2017 include the same 5 that had been funded previously, plus 2 new sites (Stanford and Massachusetts General Hospital), and Johns Hopkins was named as the new leadership center. The funding of mechanistic studies was also revamped with the creation of the "opportunity fund," a specific pot of money devoted to the development of laboratory studies to be conducted in conjunction with each clinical trial. Protocol development has been the focus since this third iteration of CoFAR was funded, and in the coming months, 2 exciting clinical trials will be launched: one focused on the use of omalizumab for the treatment of food allergy and the other on a dose-ranging approach to peanut OIT in infants and toddlers.

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