Monoclonal IgE antibodies reengineered as food allergy therapeutics

Croote D.[1], Sohail S.[1], Sanchez Rodriguez R.[1], Creeks P.[1], Wong J. J.W.[1], Boismenu R.[1]

[1] IgGenix, Inc. South San Francisco, CA 94080

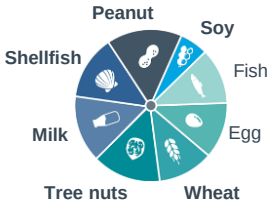
Background

Circulating B cells producing IgE antibodies are extremely rare, even in allergic individuals. This technical challenge has hindered our understanding of how monoclonal IgE antibodies recognize epitopes on allergens and mediate allergic disease in humans. Furthermore, these allergen-specific IgE antibodies, which often exhibit high levels of somatic hypermutation consistent with high-affinity binding interactions, may serve as an untapped resource for therapeutic and diagnostic development in the field of allergy.

scRNA-seq for IgE antibody discovery

We leveraged our advanced discovery platform [1] based on single-cell RNA-sequencing (scRNA-seq) to identify high-affinity human monoclonal IgE antibodies specific to a diverse range of food and non-food allergens from large numbers of allergic individuals. Discovered IgE antibodies were reengineered as monoclonal IgG4 antibodies for the development of antibody-based therapeutics capable of blocking the interaction of endogenous IgE with allergen and therefore inhibiting the allergic cascade.

Fig. 1. High level overview of our platform. Extremely rare circulating IgE-producing B cells are isolated from the blood of individuals with allergies (left) and scRNA-seq is used to recover the full-length, paired heavy and light chain sequences comprising monoclonal IgE antibodies (center). These IgE antibodies are then re-engineered such that they retain their allergen-specific IgE variable regions (Fv) but have the IgE Fc replaced with an IgG4 Fc.



- **Molds**
- **Dust mite**
- **Cat**
- **Dog**
- **Pollens**
- Medication
- Vaccines
- Venoms

Fig. 2. Our unbiased scRNA-seq discovery approach enables the isolation of monoclonal human IgE antibodies specific to any allergen humans mount an IgE response against. The “big 8” food allergens (left) and major groups of nonfood allergens (right) are shown. Allergens for which we has discovered antibodies are bolded.

Convergent evolution of peanut-specific monoclonal IgE antibodies

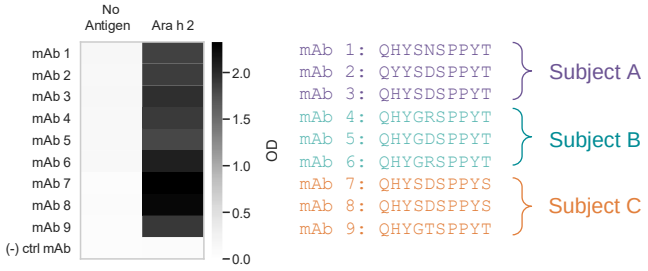


Fig. 3. Left: Convergent evolution whereby three unrelated peanut-allergic individuals each recombined three highly similar antibody sequences that all bind the immunodominant peanut allergen Ara h 2. Darker indicates stronger binding by ELISA. Center-right: Light chain CDR3 sequences, colored by individual, depict extremely high sequence similarity.

Monoclonal IgE antibody specificity recapitulates clinical tree nut coallergy

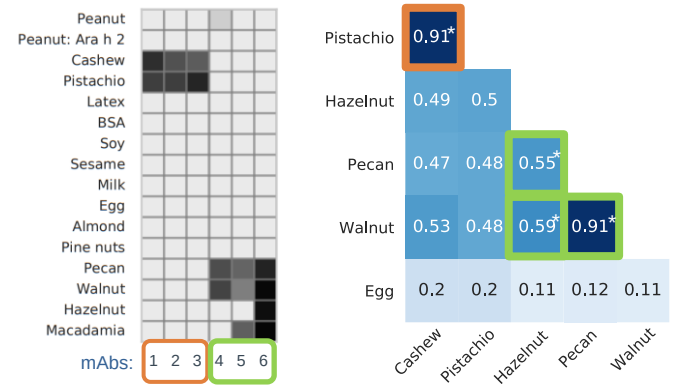


Fig. 4. Left: ELISA heatmap depicting six monoclonal IgE antibodies (mAbs) discovered from tree nut allergic donors (columns) binding to allergen extracts (rows). Darker indicates stronger binding. mAbs 1-3 bind cashew and pistachio while mAbs 4-6 bind pecan and walnut; mAb 5 and 6 also bind macadamia while mAb 6 additionally binds hazelnut. Right: Concurrence of food allergies in allergic subjects by Jaccard similarity coefficient (a coefficient of 1 equals perfect overlap). Figure created from data in [2]. * FDR adjusted p-value < 0.05

Evidence for *in vivo* competition between IgE and IgG4 in allergic individuals

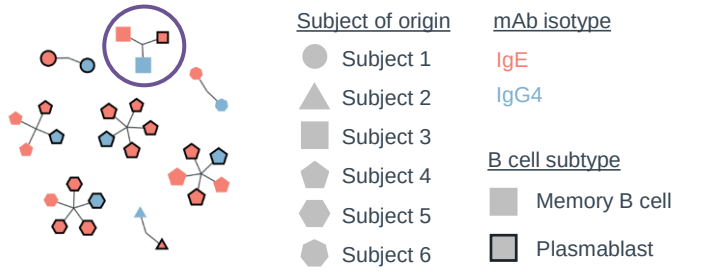


Fig. 5. Examples of B cell clonal families that demonstrate *in vivo* competition between IgE and IgG4 antibodies. Each point represents a B cell, colored by the isotype of the antibody it produces. B cells in a clonal family are connected in a network. Shape corresponds to subject of origin and a dark outline indicates that cell is a plasmablast rather than a memory B cell.

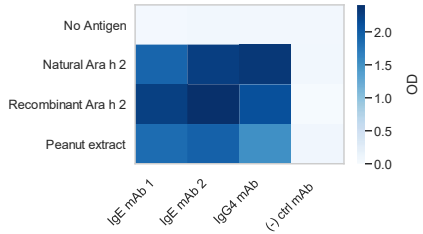


Fig. 6. ELISA demonstrating that IgE and IgG4 mAbs from the same clonal family exhibit identical allergen specificities. The three antibodies in the purple clonal family circled in Fig. 5 were run against peanut extract as well as recombinant and natural Ara h 2. No antigen wells and a non-peanut-binding mAb served as negative controls.

Conclusions

scRNA-seq is a powerful approach for isolating and characterizing rare single B cells that produce IgE antibodies. These IgE antibodies provide novel insights into allergen binding and serve as a promising starting point for the development of safe, efficacious, and convenient IgG-based therapeutics that, by design, would bypass the long onset of action and high-rate of adverse events experienced by individuals undergoing allergen-specific immunotherapies.

References & Declaration

1. Croote, D., et al. *Science* 362.6420 (2018).
2. Andorf, S., et al. *JACI: In Practice* 5.5 (2017).

Conflict of interest: all authors are employees of IgGenix, Inc.