Unbiased Single Cell Sequencing of IgE-producing B cells Yields High-Affinity Human Antibodies to the Cat Allergen Fel d 1

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Rationale

Individuals with food or environmental allergies have evolved potent IgE responses to otherwise innocuous antigens. The B cells that produce these IgE antibodies are rare, however, and the technical challenge of isolating them has impeded progress toward a molecular understanding of allergen recognition and slowed the pace of therapeutic development.

Methods

IgGenix applied its state-of-the-art single-cell RNA-sequencing discovery engine to capture extremely rare human B cells expressing IgE antibodies from peripheral blood of individuals with food and nonfood allergies [1]. IgG antibodies, designed to block the interaction of endogenous IgE with allergen and therefore prevent type I hypersensitivity reactions, were engineered from these human IgE antibodies with a IgG4 Fc and assessed for their specificity and affinity to major allergens, including the cat allergen Fel d 1.

Fig. 1. High level overview of the IgGenix SeqSifter platform. Extremely rare 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 IgG Fc.

Results

- **Sesame**
- **Cat**
- **Dog**
- **Molds**
- **Dust mite**
- **Grasses**
- **Trees**
- **Weeds**
- Medication
- Vaccines
- Venoms

Fig. 2. Our unbiased scRNA-seq discovery approach can isolate monoclonal human IgE antibodies specific to any allergen humans mount an IgE response against. The major food and nonfood allergens are shown. Allergens for which we have discovered monoclonal antibodies (mAbs) are bolded.

We incidentally discovered 9 Fel d 1 specific antibodies from distinct food allergic donors that also happened to be cat allergic. We characterized antibodies by affinity measurement and epitope binning, finding that they compared favorably with other published Fel d 1 antibodies.

High affinity, allergen-specific mAbs derived from human allergic individuals

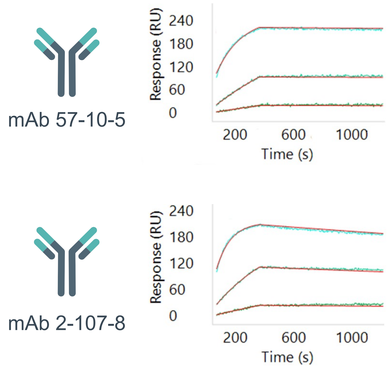

Fig. 3. Examples of IgG mAbs re-engineered from human IgE mAbs that bind to distinct epitopes on Fel d 1 with subnanomolar affinity.

Top: mAb 57-10-5 was isolated from a peanut and shellfish allergic subject that was also apparently allergic to cat.

Bottom: mAb 2-107-8 was isolated from a shellfish-allergic subject also allergic to cat.

Unbiased discovery from allergic individuals with various plasma IgE titers

IgE titer [kU/L or kUA/L]	Subject of origin for mAb 2-107-8	Subject of origin for mAb 57-10-5
Total	232	1801
Shrimp	64	143
Peanut	N/A	55
Egg	N/A	4
Cat Dander	4.2	Not tested
Fel d 1	3.9	Not tested

Fig. 5. Center: Fel d 1 specific mAbs were isolated despite this subject's much higher shellfish IgE titer, demonstrating the sensitivity of the SeqSifter platform. Right: Fel d 1-specific mAbs were isolated despite this atopic subject not mentioning their cat allergy to us, highlighting the versatility of an unbiased discovery approach.

Conclusions

An unbiased discovery approach using IgGenix's optimized scRNA-seq platform yields antibodies to a wide range of allergens. Because these antibodies are high-affinity and of human origin, they serve as promising leads for developing therapeutics that may have superior efficacy, safety, and faster onset of action compared with other Fel d 1 antibodies in development.

References & Declaration

1. Croote, D., et al. *Science* 362.6420 (2018).

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