High-affinity allergen-specific human antibodies cloned from single IgE B cell transcriptomes

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Immunoglobulin E (IgE) antibodies protect against helminth infections but can also cause life-threatening allergic reactions. Despite their role in human health, the cells that produce these antibodies are rarely observed and remain enigmatic. We isolated single IgE B cells from individuals with food allergies and used single-cell RNA sequencing to elucidate the gene expression and splicing patterns unique to these cells. We identified a surprising example of convergent evolution in which IgE antibodies underwent identical gene rearrangements in unrelated individuals. Through the acquisition of variable region mutations, these IgE antibodies gained high affinity and unexpected cross-reactivity to the clinically important peanut allergens Ara h 2 and Ara h 3. These findings provide insight into IgE B cell transcriptomics and enable biochemical dissection of this antibody class.

Although the immunoglobulin E (IgE) antibody class is the least abundant of all isotypes in humans, it plays an important role in host defense against parasitic worm infections (1). It can also become misdirected toward otherwise harmless antigens, as in the case of food allergies, where the recognition of allergenic food proteins by IgE antibodies can lead to symptoms ranging from urticaria to potentially fatal anaphylaxis. Despite their central role in immunity and allergic disease, human IgE antibodies are scarce and remain poorly characterized (2). Recent studies have inferred IgE B cell characteristics and origins (3, 4) and have described clonal families to which IgE antibodies belong (5). However, none have successfully isolated single IgE-producing cells or the paired heavy and light chain sequences that constitute individual IgE antibodies, leaving unanswered questions regarding the functional properties of such antibodies, the transcriptional programs of these cells, and the degree to which these features are shared across individuals. Here, we report the successful isolation and transcriptomic characterization of single IgE and IgG4 B cells from humans.

We performed plate-based single-cell RNA sequencing (scRNA-seq) on B cells isolated from peripheral blood of six food-allergic individuals (Fig. 1A). We used a simple fluorescence-activated cell sorting (FACS) strategy (fig. S2 and supplementary materials) that prioritized the capture of single B cells with surface IgE; we also included B cells of other isotypes for comparison. The isotype identity of each B cell was determined post hoc using the bioinformatic assembly of its heavy chain sequence from scRNA-seq reads. This allowed us to sacrifice specificity and capture IgE B cells with high sensitivity while avoiding stringent FACS gate purity requirements or the need for complex gating schemes. In total, 973 B cells were analyzed, of which 89 were IgE. We were unable to purify useful numbers of such cells from nonallergic controls.

Principal components analysis of normalized gene expression (fig. S3 and supplementary materials) separated B cells into two distinct clusters identifiable as plasmablasts (PBs) and naive/memory B cells (Fig. 1, B and C). PBs expressed PRDM1, XBP1, and IRF4, which encode the triad of transcription factors that drive plasma cell differentiation (6). In contrast, naive/memory B cells expressed IRF8, which encodes a transcription factor that antagonizes the PB fate (7), as well as MS4A1, which encodes the canonical mature B cell surface marker CD20. Additional FACS and gene expression data corroborated these B cell subsets (fig. S4).

Circulating IgE B cells overwhelmingly belonged to the PB subset (Fig. 1D and fig. S5A), which is in contrast to the other isotypes but consistent with the preferential differentiation of IgE B cells into PBs observed in mice (8). Notably, we found that the number of circulating IgE B cells for each individual correlated with total plasma IgE levels (fig. S1C). A similar phenomenon has been noted in atopic individuals and individuals with hyper-IgE syndrome (9).

Across all individuals, the 89 IgE antibodies we found varied widely in antibody heavy chain variable region (VH) gene usage as well as mutation frequency (Fig. 2A). They also varied in VH and light chain variable region (VL) complementarity-determining region 3 (CDR3) lengths (fig. S6A). There was moderate correlation between the VH and VL mutation frequency within single cells (fig. S6B), with evidence of selection via an enrichment of replacement mutations relative to silent mutations in VH and VL CDRs (fig. S6C). Relative to other isotypes, IgE B cells had a similar distribution of VH mutation frequency, use of λ versus κ light chains, and VH V and J gene usage (fig. S6, D to F).

A host of major histocompatibility complex (MHC) genes were robustly up-regulated in IgE PBs relative to PBs of other isotypes (Fig. 2B),
suggesting a more immature transcriptional program given the loss of MHC class II during the maturation of PBs to plasma cells (10). FCER2, which encodes the low-affinity IgE receptor CD23, was also highly up-regulated and coexpressed with ADAM10 in 30% of IgE PBs, indicating that a subset of IgE PBs may secrete soluble CD23 (II). LAPTM5, which encodes a negative regulator of B cell activation and antibody production (12), was also up-regulated. Down-regulated genes included LGALS1, which supports plasma cell survival (13), and those encoding the S100 proteins S100A4, S100A6, and S100A10, which may indicate reduced proliferative and survival signaling (14, 15). One of the most significantly down-regulated genes in IgE PBs encodes spleen-associated tyrosine kinase (SYK), which plays an essential role in B cell development, activation, survival, and differentiation (16). Thus, the IgE PB cell state is immature relative to other PBs with weakened activation, proliferation, and survival capacity. This suggests a potential mechanism for the short-lived IgE PB phenotype described in murine models of allergy (17).

Human IgE B cells belonging to the naïve/memory subset were deficient in immunoglobulin heavy chain membrane IgE (mIgE) transcripts, as evidenced by a lack of membrane exon splicing relative to other common isotypes. Furthermore, membrane exon splicing was detected in significantly fewer IgE PBs than non-IgE PBs (Fig. 2, C and D). The lack of mature mIgE transcripts, which could be explained by atypical polyadenylation signals that lead to poor processing of pre-mRNA (I8), is consistent with low IgE B cell receptor levels measured by others (J) and low relative IgE surface protein levels we observed by FACS. Indeed, mIgE surface protein levels on IgE B cells did not exceed those of some non-IgE B cells, which presumably display surface IgE as a result of CD23-mediated capture (fig. S2B).

By clustering cells into clonal families (CFs) according to the similarity of their antibody VH sequences (I9), we were able to observe elements of classical germinal center phenomena such as somatic hypermutation, class switching, and fate determination (Fig. 3). Only 49 cells formed CFs with multiple members (fig. S5B), which was unsurprising given the vast diversity of potential immunoglobulin gene rearrangements. Overall, these CFs contained two to six sequences, had variable isotype membership, and had a comprehensive distribution of VH mutation frequency. Four CFs illustrated the two possible B cell differentiation pathways in that they contained both PBs and memory B cells, whereas other CFs contained cells belonging to multiple isotypes. Notably, we also found that in contrast to other isotypes, IgE and IgG4 showed higher proportional membership in CFs (fig. S5C).

Surprisingly, we identified one CF (CF1) comprising cells belonging to multiple individuals: Three were IgE PBs from individual PA12, and three were IgE PBs from individual PA13 (Fig. 3). The antibodies produced by these six cells were highly similar in VH and VL sequences (fig. 4A and fig. S7, A and B), and all used the IGKV3-30*18 and IGHJ6*02 VH genes as well as the IGKV3-20*01 and IGKJ2*01 VL genes. These antibodies were also among the most mutated of all class-switched antibodies in our dataset and were enriched in replacement mutations within the VH and VL CDRs (fig. S7, C and D).

We cloned and expressed the six IgE antibodies belonging to this convergent CF in order to assess whether they bind the natural forms of the major allergenic peanut (Arachis hypogaea) proteins Ara h 1, Ara h 2, or Ara h 3. Surprisingly, all six antibodies were cross-reactive: They bound strongly to Ara h 2, moderately to Ara h 3, and very weakly to Ara h 1 (Fig. 4B). Furthermore, these antibodies have high affinity; dissociation constants determined by biosensor interferometry for Ara h 2 and Ara h 3 were as low as picomolar and subnanomolar, respectively (Fig. 4, C and D, and fig. S8). These affinities are comparable to some of the highest-affinity native human antibodies against pathogens such as HIV, influenza, and malaria (20–22).

We also cloned and expressed eight engineered variants of IgE antibody PA13P1H08 to assess the effects of VH and VL mutations on allergen binding. Retaining the actual VH while swapping the VL with another x VL from an antibody without peanut allergen specificity abrogated binding to both allergenic proteins, whereas reverting both VH and VL to the inferred naïve sequences (fig. S7, E to G) largely eliminated Ara h 3 binding and markedly reduced Ara h 2 affinity (Fig. 4D and fig. S8C). Reverting only the VH or VL reduced the affinity to Ara h 2 and Ara h 3, but disproportionately. We also found a synergistic contribution of VH mutations to affinity through independent reversion of the VH CDR1, CDR2,
CDR3, and framework regions. Interestingly, reversion of the VH CDR2 increased Ara h 3 affinity while only marginally decreasing Ara h 2 affinity. Thus, although the inferred naïve antibody is capable of binding the most clinically relevant peanut allergen Ara h 2 (23), mutations in both VH and VL are necessary to produce the high-affinity and cross-reactive antibodies that we found in circulating IgE PBs of unrelated individuals.

We also cloned and expressed antibodies from two other CFs. CF2 contained three IgE PBs from individual PA16 (two of which were identical), but these antibodies did not bind Ara h 1, 2, or 3, which was unsurprising given that this individual had low plasma peanut-specific IgE levels as well as IgE specific to other allergens (fig. S1). In contrast, CF3 contained an IgG PB (PA15P1D05) and IgG4 PB (PA15P1D12) from individual PA15. These antibodies did not bind Ara h 1 appreciably, but bound Ara h 3 with nanomolar affinity and Ara h 2 with subnanomolar affinity (fig. S8). Notably, these two antibodies used the same VL V gene and a highly similar VH V gene (IGHV3-30-3*01) as the six convergent antibodies of CF1.

Our transcriptomic characterization of circulating human IgE B cells suggests that an immature IgE PB gene expression program indicative of weakened activation, proliferation, and survival capacity contributes to the short-lived phenotype of these cells. Additionally, the absence of miRNA transcription expression supports the hypothesis that impaired membrane IgE expression compromises IgE B cell entry into the memory compartment and/or memory B cell survival, therefore causing the scarring of circulating memory IgE B cells in vivo. These results show that the human IgE system shares many important features with that of the mouse. Studies of miR signaling and IgE memory in murine models of allergy (24, 25) are therefore likely relevant for human disease.

Isolating single IgE and IgG4 B cells also provides insight into the antibodies they produce. We discovered a striking case of antibody convergence, where two unrelated individuals produced high-affinity cross-reactive peanut-specific IgE antibodies comprising identical gene rearrangements within respective VHs and VLs. Convergent antibody evolution is believed to occur in response to a number of pathogens such as influenza (26) and HIV (22). Although our results offer a single additional example, another study of peanut-allergic individuals (27) reported IgE antibodies that used identical V and J genes and shared at least 70% CDR3 identity with one or more of the six convergent antibodies in our dataset (fig. S9).

We discovered high-affinity IgE antibodies with cross-reactivity to two major peanut allergens and demonstrated that these properties originated from the acquisition of mutations within the VH and VL. Interestingly, although Ara h 2 and Ara h 3 belong to two distinct protein families, cross-inhibition experiments with purified allergens and plasma IgE have shown that this cross-reactivity may be common within peanut-allergic individuals (28). We also found an example within one individual of in vivo competition between peanut-specific IgE and IgG4 antibodies. Further study of such processes has the potential to increase our understanding of the contribution of IgG4 to the reduced clinical allergy reactivity that accompanies immunotherapy and early allergen exposure (29). Lastly, we anticipate that these antibodies or engineered variants could be used as therapeutic agents. Recent clinical results have shown that engineered-allergen-specific IgG antibodies provide effective treatment for cat allergies, perhaps by outcompeting native IgE for antigen (30).

REFERENCES AND NOTES
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SUPPLEMENTARY MATERIALS

www.sciencemag.org/content/362/6420/1306/suppl/DC1
Materials and Methods
Figs. S1 to S9
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References (31–44)

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IgE B cells unmasked

Immunoglobulin E (IgE) antibodies play a central role in immune responses against helminth and protozoan parasites; however, they also contribute to allergies. IgE antibodies (and the B cells generating them) are rare and thus poorly characterized. Croote et al. performed single-cell RNA sequencing of peripheral blood B cells from patients with peanut allergies and delineated each cell’s gene expression, splice variants, and antibody sequences (see the Perspective by Gould and Ramadani). Unlike other isotypes, circulating IgE B cells were mostly immature plasmablasts. Surprisingly, certain IgE antibodies manifested identical gene rearrangements in unrelated individuals. These IgE antibodies showed high affinity and unexpected cross-reactivity to peanut allergens.

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