

therapy with multiple bnAbs is also being explored as a way to overcome naturally circulating escape variants (Mendoza et al., 2018). The authors show that their approach is amenable to selection by multiple bnAbs and that the results generally add up to the sum of the individual parts. Again, this supports the generalizability of the approach and the potential to assess synergy or competition in a combination therapy setting.

The approach described by Dingens et al. (2019) provides the HIV research community with a valuable reference point for comparing bnAbs and a benchmark for newly identified bnAbs. The current study was accomplished using a single strain of HIV, BG505, and work remains to be done on additional strains to determine the robustness of the analysis and the most critical escape residues across a wide array of HIV strains. The

game of chess is not over, but we now have a much deeper understanding of the moves our opponent is likely to make in response to bnAb attacks.

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The Secret Life of IgE-Producing Cells

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IgE antibodies are essential mediators of allergies. In a recent study in *Science*, Croote et al. (2018) characterize IgE cells isolated from individuals allergic to peanuts. Their findings provide insight into the differentiation of IgE cells in humans and have implications for our understanding of allergic disease.

Changes in lifestyle and the environment have fueled an increase in allergic diseases, including food allergies, which disproportionately affect children. Among food allergies, peanut allergy is associated with severe reactions and long-term persistence in the majority of affected individuals. Allergies are mediated by immunoglobulin E (IgE) antibodies recognizing environmental or food proteins (Oettgen, 2016). IgE antibodies bind to high-affinity FcεR1 receptors on the surface of mast cells. Upon crosslinking by allergens, IgE antibodies induce mast cell degranulation and the rapid release of potent inflammatory mediators. To be

pathogenic, allergen-specific IgE needs to bind the allergen with high affinity and reach a critical density on the mast cell surface. The cellular processes that generate high-affinity pathogenic IgE are poorly understood. IgE concentration in plasma is extremely low, and IgE-producing cells are scarce. In addition, IgE antibodies bind to non-IgE B lymphocytes through the FcεRII receptor (CD23), confusing the detection of the few true IgE cells. The study of IgE cells is thus particularly challenging in spite of unequivocal evidence of circulating human IgE-producing cells. In breakthrough work, Croote et al. (2018) report the

characterization of single human IgE cells as plasmablasts expressing cross-reactive high-affinity antibodies belonging to convergent clonal families.

Mouse studies have provided a framework for understanding and modeling IgE responses (He et al., 2015). In mice, B cells follow a unique pathway to generate high-affinity IgE antibodies. IgE-expressing B cells are present in germinal centers (GCs), but these cells quickly exit the GC reaction and do not contribute to the high-affinity memory IgE response. Instead, most mouse IgE cells are plasma cells (Erazo et al., 2007). Nevertheless, IgE antibodies



undergo affinity maturation, and IgE memory forms. The secret to making high-affinity IgE is sequential switching, a mechanism whereby B cells undergo two recombination steps, the first one involving a switch from IgM to IgG and the second involving a switch from IgG to IgE. Sequential switching occurs predominantly through an IgG1 intermediate form in mice and through IgG1 and IgG4 intermediates in humans. Mice genetically deficient in class switching to IgG1 produce IgE after immunization but cannot mount high-affinity IgE responses. In recall responses, a population of IgG1 memory cells expressing the transcription factor *Zbtb20* generates high-affinity IgE plasma cells (He et al., 2017).

Several lines of evidence are compatible with a role for sequential switching in human IgE responses. Longitudinal studies have found that allergen-specific IgG responses precede and accompany IgE allergic sensitization (Hofmaier et al., 2015). Sequencing of the switch region of IgE-encoding genes has consistently detected molecular footprints of sequential switching (Berkowska et al., 2014). High-throughput comparison of the human immunoglobulin heavy chain repertoires determined that clonal lineages of IgE are predominantly related to IgG1 lineages (Looney et al., 2016). Through phenotypic and repertoire analyses of IgE-expressing cells at the single-cell level, Croote et al. (2018) now conclusively demonstrate that IgE plasmablasts with a history of affinity maturation, and not IgE memory cells, are the predominant circulating IgE cell type in food-allergic patients.

To optimize the isolation of circulating IgE cells from individuals afflicted by peanut allergy, Croote et al. (2018) used a low-stringency flow-cytometry gating that identified putative IgE cells as CD19⁺IgE⁺IgM[−]IgG[−] cells. IgE⁺ B cells and other B cells were sorted and processed according to a modified Smart-Seq2 protocol. Of 1,165 cells sequenced, they analyzed 973 cells that had productive heavy- and light-chain V(D)J genes. Only 89 cells were true IgE cells, and these corresponded to 29.5% of cells sorted as IgE⁺, illustrating the difficulty of identifying true IgE cells. Principal-component analysis identified a plasmablast cluster and a naive and/or memory B cell cluster. IgE cells were highly enriched in the plasmablast cluster (81 of 89 cells) and were char-

acterized by expression of the transcriptional regulators PRDM1, XBP1, and IRF4. The remaining 8 IgE cells grouped with the naive and/or memory cell cluster that differentially expressed IRF8 and CD20 (MS4A1). The analysis of exon transcripts across the IgE constant-region gene *IGH*E demonstrated the predominance of the secreted form of IgE over the membrane form, which has been attributed to inefficient polyadenylation of the membrane transcript. Analysis of the clonal repertoire demonstrated that the genes encoding IgE antibodies used a variety of V and J segments, displayed mutation rates similar to those of other B cells, and demonstrated a higher frequency of replacement than silent mutations, an indication of affinity maturation (Croote et al., 2018).

Through the analysis of clonal families among all 973 B cell clones, Croote et al. (2018) found that only 49 formed families, and among all isotypes, IgE and IgG4 cells were the most highly represented in clonal families. Interestingly, one clonal family, CF1, contained three IgE members from one patient and three IgE members from an unrelated patient. Recombinant IgG1 antibodies expressing the antigen binding sites of the six CF1 clones recognized the most clinically relevant peanut allergen, Ara h2, with high affinity and cross-reacted with Ara h3. To test the importance of the mutated residues in antigen binding, Croote et al. (2018) reverted both heavy- and light-chain sequences to the germline in one of the Ara-h2-specific antibodies. The germline antibody no longer bound Ara h2 or Ara h3 with high affinity. The results demonstrate the importance of the mutated residues in IgE antigen binding and suggest an antibody repertoire restriction in Ara h2 recognition. Interestingly, by mining the sequence data from a high-throughput study of B cell receptor repertoires from peanut-allergic subjects (Kiyotani et al., 2018), Croote et al. (2018) identified three IgE clonotypes with high similarity to the CF1 family.

The findings of Croote et al. (2018) suggest that IgE cell differentiation is similar between humans and mice in a number of ways. Most circulating human IgE cells identified were plasmablasts bearing molecular signatures of affinity maturation. Affinity maturation presumes a GC phase of the ancestors of human IgE plasma cells, which could have occurred in pre-

cursor IgG cells. The low frequency of IgE memory cells is also reminiscent of the virtual absence of IgE memory cells in mice. However, studies have reported human IgE⁺ memory cells with low membrane IgE expression and low CD79b expression in allergic individuals (Berkowska et al., 2014), though it is unclear whether these atypical IgE memory cells are functional. Given that the number of single human IgE cells characterized so far is very small, their overall diversity might not yet be fully appreciated until data from more studies become available.

An important logistical question for human IgE studies is whether circulating IgE cells reflect the disease-relevant IgE antibody pool. The detection of peanut-specific IgE plasmablasts in peanut-allergic patients argues that sampling peripheral blood is a valid approach for allergies with systemic effects. For localized allergies such as rhinitis, the affected tissue could be a better source of allergen-specific IgE cells, and this remains to be determined.

Our understanding of B cell activation and differentiation during development and later persistence or resolution of human IgE-mediated allergic diseases is still very fragmented. The work by Croote et al. (2018) clarifies important aspects of the mysterious IgE B cells (Figure 1). On the basis of the current knowledge of human and mouse IgE responses, it is plausible that human allergic responses are sustained by high-affinity IgE plasma cell and IgG memory cell precursors, given that the existence of functional human IgE memory cells is still in doubt. The lifespan of IgE plasma cells and the conditions in which new IgE plasma cells are generated are therefore critical questions in understanding allergies and identifying nodes for therapeutic intervention. Short-lived and long-lived humoral IgE responses have been described in humans and mice. Seasonal pollen allergies are accompanied by bursts of pollen-specific serum IgE, presumably as a result of *de novo* formation of short-lived IgE plasma cells. In the case of perennial allergens, persistent IgE could be attributed to long-lived IgE plasma cells or short-lived IgE plasma cells continuously generated form a memory reservoir. The fact that most IgE cells identified in the Croote et al. (2018) study were plasmablasts is consistent with a continuous generation

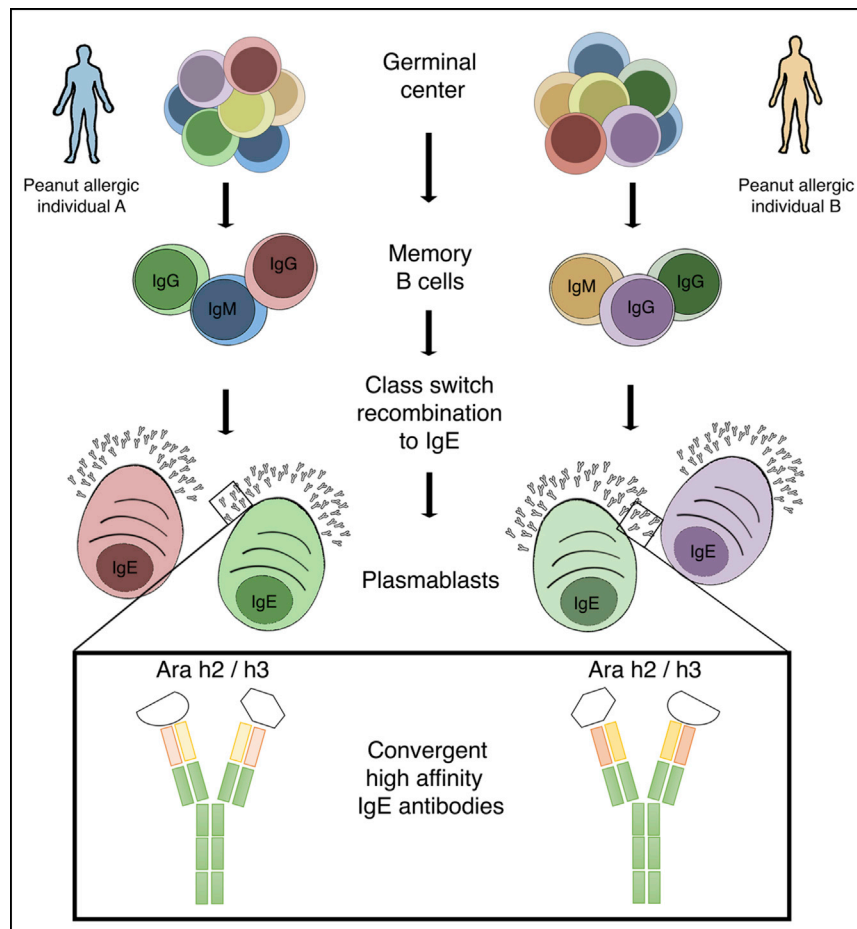


Figure 1. Model for the Formation of High-Affinity Peanut-Specific IgE in Peanut Allergy

The development of peanut allergy in humans (represented here by individuals A and B) involves a GC reaction and the differentiation of memory B cells containing high-affinity clones. During re-exposure to peanut antigens, high-affinity memory B cell clones presumably of the IgG type undergo class-switch recombination to IgE and plasma cell differentiation. Croote et al. (2018) described that in peanut-allergic individuals, most circulating IgE cells are affinity matured plasmablasts and that unrelated individuals harbor convergent and cross-reactive IgE clones that bind the disease-relevant peanut allergens Ara h2 and Ara h1 with high affinity. Their findings are represented in the context of a current model of IgE cell differentiation.

of IgE plasma cells in food allergy. In addition, if these plasmablasts originate from non-IgE memory cells, then inhibiting class-switch recombination to IgE, an interleukin-4 (IL-4)- and IL-13-dependent process, should decrease allergen-specific IgE. Interestingly, treatment of atopic dermatitis patients with an antagonist anti-IL-4R α antibody resulted in a consid-

erable reduction of serum IgE (Beck et al., 2014), arguing that a large portion of circulating IgE in these patients derives from short-lived IgE plasma cells generated in recent class-switching events. Finally, the molecular characterization of the binding of IgE antibodies to disease-relevant allergens could help in the design of molecules that, by competing for

allergen binding, would alleviate IgE-mediated allergic disease.

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