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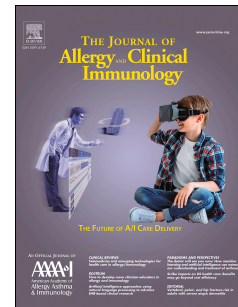
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IgG+ memory B cells: friends or foes in allergic disease?

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Specific allergen immunotherapy has long been used empirically¹, but the benefits to patients have yet to be fully rationalised. In a recent paper in *JACI*², Hoof *et al.* present evidence for a stepwise process in which allergen-specific IgG+ memory B cells undergo affinity maturation before switching to IgE and secreting high-affinity IgE antibodies against multiple allergen epitopes, as required to mediate the allergic response³. They show that the resulting IgE-secreting cells produce a short burst of allergen-specific IgE antibodies of high affinity, which are clearly pathogenic in allergy, and then die. Their work illuminates the intermediate steps between the development of IgG+ memory B cells, and the generation of the clonally related IgE-secreting cells (Fig. 1).

This study was carried out in the context of sub-lingual allergen immunotherapy (SLIT) of grass pollen allergic rhinitis patients. It is well known that the concentration of IgG4, a minor subclass of IgG, is dramatically up-regulated (up to 100-fold) during the dose escalation phase of specific allergen immunotherapy. Paradoxically, it is the “blocking” activity of IgG4, the ability to compete with IgE for antigen binding, that is most often associated with the induction tolerance to grass pollen, which has been emphasised in the past⁴. The opposite effects of IgG-memory B cells in the generation of pathogenic IgEs may appear to be inconsistent. Below we comment on the findings of Hoof *et al.*, and suggest how to resolve

32 this “paradox”. The bottom line is that IgG is poised between tolerogenic and pathogenic
33 functions in the IgE adaptive immune response.

34
35 The authors used next generation sequencing (NGS) of 21 subjects with grass pollen allergy,
36 undergoing sub-lingual immunotherapy (SLIT) to identify clonal relationships between IgG
37 and IgE at the peak of IgE synthesis after 4 weeks of treatment. About 20% of the IgG (and
38 less frequently IgA) clones were found to be clonally related to IgE (Fig. 1). These cells were
39 termed IgG_E B cells. This was followed up in a 12-month longitudinal study of 3 subjects.
40 Samples of blood were collected at intervals to examine changes in the B cell repertoire.
41 Predictably, most of IgG_E clones found at 4 weeks and thereafter were also found, although
42 at far lower frequency, at baseline. Moreover, the IgE and IgG_E clones increased and
43 decreased in parallel, and the levels of somatic hypermutation (SHM), reflecting affinity
44 maturation, were comparable. The number of both IgG_E and other IgG clones decreased to
45 half the peak values by day 7 and persisted thereafter. This suggested pre-commitment to a
46 repertoire of antibodies established *in vivo* by SHM and selection of IgG precursors of IgE
47 with high affinity for antigen prior to indirect/sequential switching from IgG to IgE (IgM →
48 IgG → IgE), without further SHM in IgE. There are many other reports of persistent IgE
49 memory responses, but this study provides new evidence for an undoubtedly important
50 pathogenetic mechanism.

51
52 Antibodies of any antibody class or subclass, in principle, should be able to compete with IgE
53 at sufficient concentration. However, IgG4 may have more frequent opportunities to
54 accumulate somatic mutations than other IgG subclasses, due to its downstream position in
55 the immunoglobulin heavy chain locus and multiple switching events prior to IgE.
56 Accordingly, the authors demonstrate the expression of IgG_E subclasses other than IgG4 as
57 precursors of IgE in their recombinant antibodies. The subtype of IgG_E is not specified.

58
59 Indirect switching from IgG to IgE and the poor survival of the nascent IgE-switched cells is a
60 familiar scenario from mouse models of allergy⁵ and also *in vitro* studies of class switching
61 to IgE in human tonsil B cells⁶. The poor survival of the vast majority of IgE-secreting cells
62 outside of the bone marrow has been repeatedly observed by others. We speculate that this
63 mechanism evolved to reserve the most potent, but risky, IgE isotype for the occasional

exposure to antigen, as needed. This may also be advantageous by refreshing the local B cell repertoire⁷.

To compare the B cell repertoire in blood and the nasal mucosa, the authors took biopsies and samples of blood from 7 donors at baseline and after 12 months of SLIT. Although the repertoire in the nasal mucosa was skewed towards IgA, the percentage of IgG_E cells was the same in both compartments. This is compelling evidence for the rapid trafficking of the B cells in both directions between the compartments, as previously reported for IgE clones in the respiratory tract in asthma⁸. Mobilisation of the B cells may be induced by IL-4 in the Th2 environment of the nasal mucosa. The observations of Hoof *et al.* on the oral mucosa extend the field of B cell trafficking in the respiratory tract to include the oral cavity, relevant to sublingual immunotherapy. It would be of interest to look further afield and screen for possible homing factors (chemokines, adhesion factors), that may direct B cells to the nasal mucosa in rhinitis and to other target organs of allergic disease.

Next, the authors separated the B cells in blood into naïve B cells, memory B cells and plasmablasts to reveal possible differences in their contribution to IgE switching at the 4-week time point. Naïve B cells were distributed between many small clusters of IgM or IgD isotypes. Plasmablasts were of relatively large clusters of all isotypes (except IgD), shaped by clonal expansion. The memory cell repertoire contained many small clusters of all isotypes, but was dominated by a few large IgE clonotypes. These represented the IgE clones that secreted around 20% of total number of immunoglobulin transcripts, including all 5 isotypes. Up until now, there has been little evidence for a population of IgE⁺ memory cells. The abundant IgE plasmablast cells recovered in the memory cell compartment may be transient survivors in the long progression of affinity maturation and selection of IgG in their trajectory to IgE.

The heterogeneity of the memory B cell compartment was further investigated by transcriptomic profiling of 93 single B cells from the week-4 memory B cell population of one subject. 85 cells exhibited the expected naïve/memory cell phenotype (CD38⁻). However, 8 IgE⁺ cells exhibited a more plasmablast character, consistent with the results (above) at the cell population level. Their presence in the memory cell population was found

to be due to their lack of expression of CD38 (used to isolate the memory cell population), despite their expression of other key plasmablast markers. This also explained the dominance of IgE transcripts in the total VH repertoire of the “memory” cell population, given the high rate of IgE secretion of plasmablasts relative to memory B cells. These cells may be similar to “pre-plasmablasts”, a prominent early stage of cell differentiation into plasma cells⁶. The results in sublingual immunotherapy are consistent with the view that repeated (or chronic) exposure of sensitive individuals may eventually focus the IgE repertoire.

For 64 single cell transcriptomes it was possible to determine the matched VH sequences and align them with the individual’s total VH repertoire. Eleven sequences aligned with IgG or IgE clonotypes. These and 5 additional sequence pairs from B cells that expressed ϵ germline gene transcripts, indicating the potential for IgE switching, were expressed as recombinant antibodies. Fourteen of the 16 antibodies exhibited high affinity binding to major grass pollen allergens. Moreover, in combination, they sensitised basophils *in vitro* to allergen-induced degranulation of basophils, providing proof of concept that they can be pathogenic in the nasal mucosa *in vivo*.

The existence of IgG_E cells opens up important new questions: What prompts IgG memory cells to become IgG_E cells rather than IgG+ plasma cells, and ultimately into short-lived IgE+ (pre-)plasmablasts? Is this tolerizing phenomenon in SLIT analogous to the tolerization induced in young children thought to be at risk of sensitisation to risky allergens (*e.g.* peanuts) by early oral exposure to the allergen?⁹ Extended exposure to allergens is known to have certain cumulative and long-term (irreversible?) consequences⁴. Could these effects be caused by the gradual down-regulation of IgE receptors, as they become saturated *in vivo* (a pro-zone effect)? Why do the IgE-switched cells differentiate exclusively into the plasma cell lineage? Could this be related to autonomous (antigen-independent) signalling from the IgE BCR¹⁰ resulting in a pause in differentiation at an early (CD27+CD38-) stage of plasma cell differentiation? Deeper investigation may further clarify the mechanisms and reveal new targets for clinical intervention in allergic disease.

References

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- 129 1. Noon L. Prophylactic inoculation against hayfever. *Lancet* 1911;177:1572–3.
- 130 2. Hoof I, Schulten V, Layhadi JA, Stranzl T, Christensen LH, Herrera de la Mata S, et al.
131 Allergen-specific IgG+ memory B cells are temporally linked to IgE memory responses.
132 *J Allergy Clin Immunol* 2019;
- 133 3. Christensen LH, Holm J, Lund G, Riise E, Lund K. Several distinct properties of the IgE
134 repertoire determine effector cell degranulation in response to allergen challenge. *J*
135 *Allergy Clin Immunol* 2008;122:298–304.
- 136 4. Shamji MH, Durham SR. Mechanisms of allergen immunotherapy for inhaled
137 allergens and predictive biomarkers. *J Allergy Clin Immunol* 2017;140:1485–98.
- 138 5. Erazo A, Kutchukhidze N, Leung M, Christ APG, Urban JF, Curotto de Lafaille MA, et al.
139 Unique maturation program of the IgE response in vivo. *Immunity* 2007;26:191–203.
- 140 6. Ramadani F, Bowen H, Upton N, Hobson PS, Chan YC, Chen J-B, et al. Ontogeny of
141 human IgE-expressing B cells and plasma cells. *Allergy* 2017;72:66–76.
- 142 7. Gould HJ, Wu Y-CB. IgE repertoire and immunological memory: compartmental
143 regulation and antibody function. *Int Immunol* 2018;30:403–12.
- 144 8. Ohm-Laursen L, Meng H, Chen J, Zhou JQ, Corrigan CJ, Gould HJ, et al. Local Clonal
145 Diversification and Dissemination of B Lymphocytes in the Human Bronchial Mucosa.
146 *Front Immunol* 2018;9:1976.
- 147 9. Toit Du G, Roberts G, Sayre PH, Bahnson HT, Radulovic S, Santos AF, et al.
148 Randomized trial of peanut consumption in infants at risk for peanut allergy. *N Engl J*
149 *Med* 2015;372:803–13.
- 150 10. Haniuda K, Fukao S, Kodama T, Hasegawa H, Kitamura D. Autonomous membrane IgE
151 signaling prevents IgE-memory formation. *Nat Immunol* 2016;17:1109–17.

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Figure 1. IgG friends and foes in the allergic response

Every B cell clone generated in the bone marrow is unique because of the enormous diversity of the B cell repertoire in an individual, which is orders of magnitude less than the potential antibody repertoire at any given time. The unique ancestral B cell clones (top of tree in A) in each individual, generally different from every other individual at this time, originates from immature B cell precursors in the bone marrow, which emigrate into the circulation. Following antigen stimulation, the cells may undergo class switching to IgG, somatic hypermutation and class switching to IgE at the sites of exposure to antigen or the local lymphoid tissue. The IgE⁺ cells inherit the specificities and affinities of the IgG⁺ cells and differentiate into IgE-secreting plasma cells (family tree in A).

The main point of this diagram is to show how class switch recombination (CSR) can generate friendly or unfriendly IgGs. Secreted IgE binds tightly to the high-affinity receptor on mast cells and basophils and antigen cross-linking of the receptors induces cell activation to cause immediate hypersensitivity and initiate allergic inflammation. Friendly IgG⁺ antibodies, secreted by B cells closely related to IgE⁺ B cells, the sisters, cousins and aunts of the IgE⁺ B cells (family tree), can compete with IgE antibodies for the free antigen, thereby preventing crosslinking of the antigen to the corresponding antigen-IgE complexes on effector cells to suppress the allergic response (B). Alternatively, unfriendly IgG_E (memory IgG clones that express antibodies of high affinity against antigen) may, in IL-4 conditions, switch to IgE in the presence of antigen thereby enhancing the allergic response (C).

