

Study of stereotyped, allergen-specific IgE sequences from specific immunotherapy participants yields new insight into mechanisms of allergy suppression



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In the July 2023 issue of the *Journal of Allergy and Clinical Immunology*, Hoh et al¹ used the techniques of single-chain fragment variable (scFv) phage display and next-generation sequencing to study the repertoires of IgE- and IgG4-bearing B cells in the blood and nasal biopsy specimens from participants with allergy treated with specific immunotherapy (SIT). They used the stereotyped antibody response to the timothy grass pollen allergen *Phleum pratense* (Phl p) 2² to gain new insight into the mechanisms by which SIT modulates the IgE response.

SIT has been used to treat allergy since 1911, when it was shown that repeated injections of crude grass pollen extract reduced immediate conjunctival grass pollen sensitivity. In 1921, serum "reagin" (identified as IgE in 1966) was found to passively transfer immediate hypersensitivity. In 1935, transfer of post-ragweed immunotherapy serum containing allergen-specific IgG and/or IgA was shown to block ragweed hypersensitivity. In 1999, it was found that 3 years of continuous SIT yielded long-term benefits.³

In spite of this century-long experience, the mechanisms underlying SIT remain unclear. Whether SIT alters the distribution, prevalence, or affinity maturation of IgE-producing B cells is unknown, as is whether SIT enhances the prevalence of allergen-specific IgG4-producing B cells. A major stumbling block to answering these questions is the vanishingly small numbers of IgE-producing B cells in the blood and tissues. They are difficult to detect in allergy-free individuals. Even after major antigen challenge, they may at best rise to only approximately 0.7% of memory B cells and approximately 0.02% of plasmablasts or plasmacytes in the blood.⁴

IgE, as is typical for immunoglobulins, is a heterodimeric protein composed of 2 heavy (H) and 2 light (L) chains.⁵ Each chain contains a constant (C) domain, that specifies antibody function, (eg, binding to Fcε receptors on basophils and mast cells) and a variable (V) domain, which contributes to antigen

recognition. Diversity is concentrated in the 3 complementarity-determining regions (CDRs) of the V domain, which are juxtaposed with the 3 CDRs of the other chain's V domain to form the antigen-binding site. Individual H chain variable domains are created by the combinatorial juxtaposition of V, diversity (D) and joining (J) gene segments. The site of their rearrangement comprises the third CDR (CDR-H3). CDRs 1 and 2 are part of the V gene segment and are thus germline encoded. V domain diversity is exponentially enhanced by exonucleolytic nibbling of terminal germline VDJ sequence and by the addition of non-templated (N) nucleotides between the rearranged V_H, D_H, and J_H gene segments, further increasing D in CDR3-H3. L chains are less diverse because they lack a D gene segment, limit terminal exonuclease loss, and typically include few if any N nucleotides between V_L and J_L. After antigen exposure, B cells can activate a process of somatic hypermutation (SHM) that allows exponential diversification of both the V_H and V_L domains. Whereas initial antigen specificity is greatly dictated by CDR-H3, which includes the D_H in its entirety, affinity maturation is typically marked by somatic mutation focused on CDRs 1 and 2, presumably to create a better "fit." scFvs contain 1 copy of a linked set of H and L V domains and thus express the antigen specificity of an antibody but not its effector function.

Screening for human antibodies that bind specific antigens was greatly facilitated by the invention of scFv phage display.⁶ V_H and V_L exons are amplified from human B cells and then expressed as random pairs on the surface of a phage, a bacterial virus. The target antigen is immobilized on a solid surface (eg, microtiter plate wells) and incubated with the collection of scFv-bearing phage representative of the immunoglobulin repertoire in the tissues from which they are derived. The antibody V domains expressed by the phage that bind to the target antigen can be sequenced to assess their genetic origin. Next-generation sequencing of reverse mRNA transcripts from antibody-producing B cells creates a library of V domains that permit identification and enumeration of allergen-binding H chains from different tissues from the same individual. Use of the proper C_H primer can yield immunoglobulin class-specific repertoires, such as Igμ or Igγ4. Using these techniques, Hoh et al¹ were able to identify scFvs that bound the allergens *Betula verrucosa* 1, *Betula verrucosa* 2, Phl p 1, Phl p 2, Phl p 5, Phl p 6, and *Der-matophagoides pteronyssinus* 1.

Neither of the aforementioned techniques provides information about the original H and L chain pairs. Thus, these approaches cannot completely recapitulate the original allergen-specific isotype repertoire. Although the random nature of N addition promotes production of widely divergent antibody repertoires, antibodies containing mostly germline sequences are often shared

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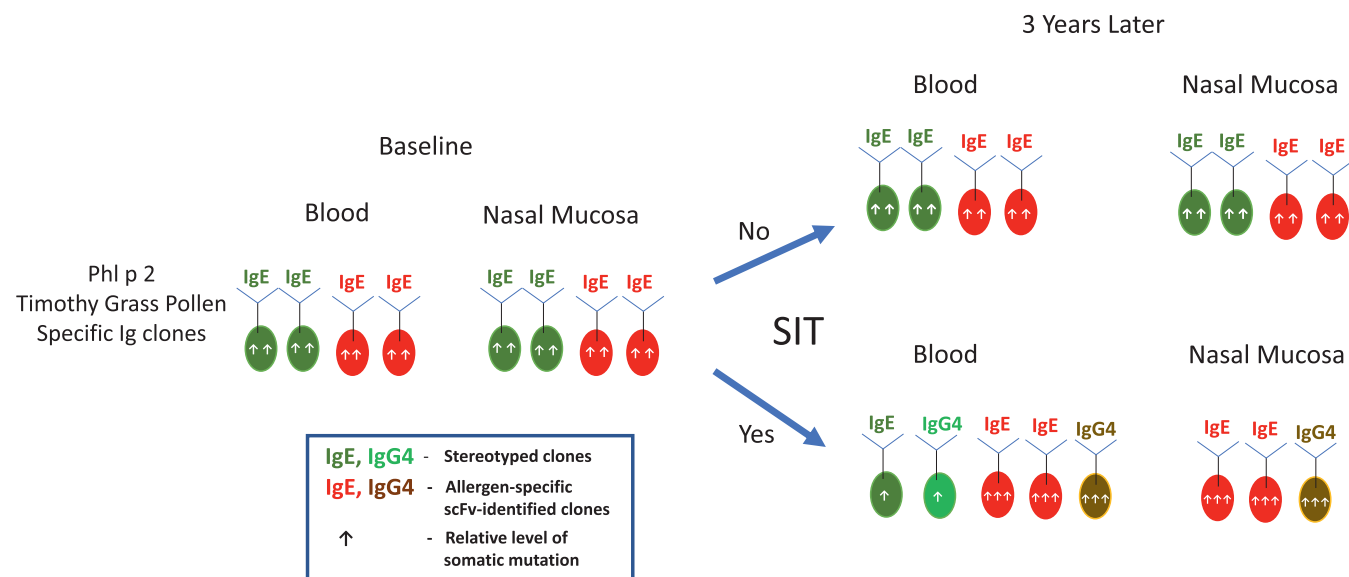


FIG 1. Before the initiation of SIT, B cells bearing IgE specific for the timothy grass pollen allergen Phl p 2 are found in the blood and the nasal mucosa of individuals with grass pollen allergy. These IgE can be identified either by a stereotyped V_H domain sequence or by panning for scFvs that bind Phl p 2. Both types of sequences contain somatic mutations in their antigen-binding sites, which is suggestive of affinity maturation following antigen exposure. After 3 years, the blood and nasal mucosa of untreated individuals with allergy continue to contain these cells. Among treated individuals with allergy, allergen-specific scFv IgE sequences from both blood and nasal tissues are more heavily mutated, suggesting continued affinity maturation. After treatment, however, B cells bearing stereotyped IgE are rare in the nasal mucosa. Further, the stereotyped IgE in the blood B cells exhibits fewer somatic mutations than at baseline, suggesting either the loss of affinity-matured, stereotyped IgE B cells, the recruitment of less-mutated IgE-bearing cells, or both. IgG4-bearing cells expressing stereotyped or allergen-specific Fv are commonly found in either the blood or nasal mucosa of SIT-treated individuals, whereas these cells are rare in untreated individuals. Hoh et al¹ hypothesize that prolonged treatment with SIT opposes the ongoing residence or survival of highly affinity-matured stereotypic IgE-bearing cells while promoting the development of B cells bearing allergen-specific IgG4. These IgG4-producing cells may be able to outcompete tissue-resident, allergen-specific IgE-bearing B cells, resulting in a reduction in symptoms among participants treated with SIT.

between individuals. Instead of an exhaustive search for all antigen-binding antibodies, an alternative approach to dissection of the development, function, and life history of antigen-specific responses relies on the identification of “convergent,” “public,” or “stereotyped” antibodies. These public antibodies can be heavily dependent on germline (ie, unmutated VDJ_H sequence).⁷

The H chains of the stereotyped anti-Phl p 2 sequences use the germline V_H gene segment IGHV4-31 or its highly related sibling IGHV4-30-4 and contain only 9 or 10 amino acids in CDR-H3, with glycine in a middle position. Hoh et al¹ found that a D/NGY ([Asp/Asn]-Gly-Tyr) motif, which is encoded by the germline D_H gene segment IgHD5-24, was common in IgE from allergen-seropositive individuals but rare in seronegative individuals. Molecular models of a solved IgE structure revealed the close proximity of these amino acids to the bound allergen Phl p 2.

After 3 years of SIT, stereotyped anti-Phl p 2 antibodies were found primarily in the blood, whereas in participants who did not undergo SIT, these antibodies were found both in nasal tissues and, at low frequency, in blood (Fig 1). The levels of Phl p 2–stereotyped IgGs were higher after SIT than at baseline. After 3 years of SIT, IgG4s bearing stereotyped or scFv sequences were detected in the blood and nasal mucosa. IgE sequences were heavily mutated in CDR-H1 and CDR-H2 before initiation of SIT, consistent with a history of affinity maturation toward allergen. Eight weeks after SIT initiation, however, IgE sequences

expressed lower levels of SHM, which is suggestive of recruitment of less–antigen-experienced B cells into the local tissue allergen response. SHM levels were increased at year 3 for scFv phage display–identified sequences, although the SHM level in stereotyped sequences remained depressed.

Although not yet definitive, use of state-of-the-art techniques has permitted Hoh et al¹ to support the view that SIT promotes the recruitment of less–affinity-matured allergen-specific IgE-producing B cells into the blood. After 3 years of SIT, these allergen-specific IgE-bearing B cells are less likely to be found in the nasal tissues than in untreated participants. This could reflect changes in tissue trafficking or survival. Finally, at 3 years, B cells bearing allergen-specific IgG4 antibodies are detectable in SIT participants but not in untreated participants. This supports the view that SIT is associated with recruitment or survival of IgG4 allergen-specific clones. These findings are encouraging and exciting, marking an important milestone. Nevertheless, much work remains to be done.

DISCLOSURE STATEMENT

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