The enigma of IgE$^+$ B-cell memory in human subjects

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Our understanding of the origin and fate of the IgE-switched B cell has been markedly improved by studies in mouse models. The immediate precursor of the IgE-switched B cell is either a relatively naive nonswitched B cell or a mature IgG-switched B cell. These 2 routes are referred to as the direct and indirect pathways, respectively. IgE responses derived from each pathway differ significantly, largely reflecting the difference in time spent in a germinal center and thus time for clonal expansion, somatic hypermutation, affinity maturation, and acquisition of a memory phenotype. The clinical and therapeutic implications for IgE responses in human subjects are still a matter of debate, largely because the immunization procedures used in the animal models are significantly different from classical atopic sensitization to allergens from pollen and mites. On the basis of the limited information available, it seems likely that these atopic IgE responses are characterized by a relatively low IgG/IgE ratio, low B-cell memory, and modest affinity maturation, which fits well with the direct switching pathway. It is still unresolved how the IgE response evolves to cover a wide epitope repertoire involving many epitopes per allergen, as well as many different allergens from a single allergen source. (J Allergy Clin Immunol 2013;131:972-6.)

Key words: *IgE, B cells, IgE$^+$ B cells, memory B cells, plasma cells, allergy, germinal center*

Abbreviations used
CDR: Complementarity determining region
GC: Germinal center
PC: Plasma cell
SHM: Somatic hypermutation
SIT: Allergen-specific immunotherapy

To understand the development of prototypic atopic allergic reactions to pollen and mites, we have to understand the peculiarities of the origin and fate of the IgE-switched B cell. Exciting new information, largely obtained in the mouse model, has been obtained since we discussed these topics almost a decade ago.[1] Here we consider recent developments in murine models of IgE activation, pathways of switching to IgE, features of IgE transcripts, the affinity and repertoire of IgE recognition of allergens, and the clinical and therapeutic implications of these characteristics of IgE in patients with allergic disease.

The new data support the notion that there are 2 roadblocks on the way to the production of allergen-specific IgE. The first is at the level of the class switch to IgE, and the second is at the level of survival of the IgE-switched B cell. It is important to stress that it is not only the switch to IgE that limits IgE production. If this was the major limiting factor, IgE responses would involve only few clones. As will be discussed in more detail below, this is not the case. Some unusual features of the IgE-switched B cell limit both its clonal expansion and its survival as a memory B cell. This has much to do with the short residence time of the IgE-switched B cell in the germinal center (GC). Combining observations in atopic subjects with these recent data from mouse models, we will argue that the atopic IgE response is made of many small clones, covering a relatively wide spectrum of epitopes on several allergens and a low IgG/IgE ratio compared with the animal models.

The discussion on the properties of B cells involved in IgE production is complicated because it is now fully accepted that the immediate precursor of the IgE-switched B cell can be either a relatively immature nonswitched (ie, IgM$^+$) B cell or a mature antigen-experienced class-switched (typically IgG positive) B cell. These 2 routes to IgE are referred to as the direct switch (IgM to IgE) and the indirect switch (IgM via IgG to IgE). IgE production through an IgG-switched B-cell precursor is in many ways similar to a conventional antibody response because the IgG-switched precursor has full access to the special features of the GC reaction (proliferation, somatic hypermutation [SHM], affinity maturation, and memory cell development). In contrast, the IgE-switched B cell is found to have a phenotype markedly different from that of the IgG-switched B cell.

In one of the recent mouse studies, it is argued that IgE produced through the indirect switching pathway is most relevant for human allergic disease, whereas IgE produced through the

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direct route is assumed to be irrelevant or even protective.[2] We argue here that atopic sensitization is caused by IgE production through the direct route.

Clarification of these issues is important for the development of preventive and therapeutic strategies. Much will depend on detailed analyses of the allergen-specific IgE response in human subjects, particularly in birth cohort studies. Transcriptomic analysis of the IgE heavy chain transcripts, both of the variable domain complementarity determining region (CDR) 3 region and the 3′ region,[3] is likely to yield important information, but the scarcity of IgE-switched B cells (or pre–plasma cells [PCs]) in human blood makes these studies challenging. Detailed longitudinal analyses of the epitope specificities of IgE and IgG antibodies in plasma by using microarrays containing allergenic proteins and allergen peptides is a complementary approach that should help us to understand the dynamics of the development of the IgE repertoire in the setting of atopy.

THE ELUSIVE IgE-SWITCHED B CELL

Circulating IgE^+ B cells are extremely rare in human subjects.[4-7] Analysis of IgE cDNA transcripts from cells in human blood indicates that most circulating IgE-producing cells are more related to PCs than to B cells (see below). The scarcity of IgE-committed memory B cells is supported by the plasma IgE antibody response on allergen injection during allergen-specific immunotherapy (SIT), which is very modest (typically 2-fold) for IgE, compared with 10- to 100-fold for IgG.[8] A lack of persistent memory IgE B cells has also been observed in 2 mouse models.[9]

In view of these data, it came as a surprise that Kelly and Butch[10] noted a selective presence of IgE^+ B cells in GCs. Although some of these cells might have stained positively because of IgE captured by the low-affinity receptor for IgE (CD23), many IgE^+ cells were $CD23^-$. Erazo et al[11] demonstrated that in immunized mice B-cell isotype switching to IgE was initiated within the GC but that IgE^+ B cells were located away from the T-cell zone. An implication from these observations is that IgE^+ B cells observed within the GC do not receive adequate T-cell help to support IgE^+ B-cell maturation, SHM, or affinity maturation. These IgE^+ B cells differentiated into PCs and left the GC rapidly once they had switched isotype to IgE, thereby not further mutating their variable gene segments.[11] The IgE response has been described as "PC-centric."[12] An inference from this study is that IgE^+ B cells predominantly exist as PCs, which explains why persistent memory IgE^+ B cells are rare. This unusual development pathway for IgE^+ B cells has important unresolved implications not only for the generation of persistent IgE^+ memory B cells available for recall response on subsequent allergen exposure but also for the accumulation of somatic mutations and affinity maturation in IgE^+ B cells.

TRACKING IgE-SWITCHED B CELLS BY USING MICE WITH TRANSGENICALLY TAGGED IgE

IgE reporter mice with an increased power to detect IgE^+ B cells have recently been generated. Talay et al[13] challenged the current thinking that IgE^+ memory B cells do not participate in GC reactions. The authors cleverly engineered mice to express green florescence protein in tandem with the membrane form of IgE under the control of the IgE promotor. With these mice, they were able to identify in 2 models (*Nippostrongylus brasiliensis* infection and a hapten-protein conjugate) IgE-switched GC B cells, memory B cells, and IgE^+ PCs with a high sensitivity and specificity. Their sensitivity was sufficient to reliably confirm the presence of IgE-switched B cells in GCs and that the number of memory B cells in spleens and draining lymph nodes was very small indeed. On adoptive transfer of these memory IgE^+ B cells and not $IgG1^+$ B cells to recipient mice lacking B cells (μMT mice), a small IgE memory response was observed. However, it appeared that these IgE^+ memory B cells did not engage further in GC reactions but were rapidly reactivated and differentiated to PCs.

Yang et al[14] generated mice that express IgE as a single transcript of yellow fluorescent protein (Venus)–tagged membrane IgE. With the increased sensitivity of the Venus-IgE detection, this group also saw IgE^+ B cells in both the GC and PC compartments. Unexpectedly, the IgE^+ PCs that developed after immunization increased expression of surface IgE, whereas IgG_1-expressing PCs showed decreased levels of surface immunoglobulin staining, which is consistent with known downregulation of surface immunoglobulin in secreting cells. This high surface IgE expression on PCs was confirmed in wild-type mice, leading the authors to argue that the membrane form of IgE transgene did not artifactually increase expression of IgE on PCs. Consistent with the emerging picture, a higher proportion of IgE^+ B cells differentiated into PCs than IgG_1^+ B cells. Despite this "premature" progression to the PC compartment by IgE^+ B cells and low numbers of IgE^+ B cells in GCs at day 14, both IgE and IgG_1 showed increased SHM at day 14 compared with that seen at day 7 after immunization. Nonetheless, IgE^+ PCs showed reduced levels of SHM compared with IgG_1^+ PCs. The technical merits of both these studies have received commentary elsewhere.[2,15] These studies clearly indicate that the phenotype of the IgE-switched B cell differs markedly from that of the IgG-switched B cell in terms of residence time in GCs, the propensity to develop into PCs, and the correlation between membrane-anchored and secreted IgE.

TRANSCRIPTOME ANALYSIS OF THE IgE HEAVY CHAIN VARIABLE DOMAIN CDR3

The case for high-affinity allergen-driven IgE in patients with allergic disease includes observations that IgE transcripts of 3 patients with grass pollen allergy showed IgE with related CDR3 and high levels of somatic mutations within the CDR, which is consistent with oligoclonal expansion of B cells.[16] On analysis of 1366 IgE transcripts from 13 children, it was noted that the patients with asthma showed evidence of more clonal diversity and higher SHM in their IgE repertoires than patients with atopic dermatitis.[17,18] IgE transcripts of parasitized subjects from Papua New Guinea showed low clonal diversity and a low frequency of somatic mutations within IgE compared with IgG_4 transcripts.[19] These results support the view that atopic dermatitis and parasitic infestations are not equivalent to the IgE response to pollen and mites. A more complete account of the cases for and against multiclonality of IgE antibodies resulting from an allergen-driven B-cell response involving antibodies with a modest level of somatic mutations in patients with atopic allergy has been reviewed elsewhere.[20,21]

AFFINITY OF IgE TO PROTEIN ALLERGENS AND ITS RELATION TO THE EFFECTOR FUNCTION OF IgE

Xiong et al[22] argue that the indirect, high-affinity route to IgE is most relevant in relation to atopic allergy. Their main argument is

that the affinity of IgE produced through the direct route is too low to be pathogenic. We want to argue that IgE produced through the direct route is the most relevant in the setting of atopic allergy. The 2 antigens used for the affinity discussion by Xiong et al[22] were both haptens: a 13-mer peptide and nitrophenyl. It is difficult to extrapolate these data to IgE antibodies elicited against classical atopic allergens from pollen and mites. All major allergens are proteins that have multiple surface epitopes, which is relevant to events that occur after initial contact with an IgE molecule on a basophil or mast cell. In addition, we know from the work of Christensen et al[23,24] that the moderate affinity of allergen-specific IgE is high enough to trigger basophils. They studied in detail the effect of IgE affinity on the allergen-induced activation of human basophils. Their data support a 2-step model for allergen-induced cross-linking of cell-bound IgE. A small fraction of IgE with an affinity of $10^9\ M^{-1}$ is sufficient to catch allergen. The resulting 1:1 IgE-allergen complex floats over the cell surface and interacts with other IgE antibodies (Fig 1).[23] These latter interactions result in cell activation, even with low-affinity antibodies (artificially generated in this study by swapping light chains). According to this model, only a small fraction of allergen-specific IgE needs to have an affinity of $10^9\ M^{-1}$. Moreover, IgE antibodies of very low affinity can still markedly contribute to the activation of effector cells in patients with allergic disease because all genuine protein allergens have multiple IgE binding sites. However, although high-affinity allergen-specific IgE is not essential for activation of basophils in patients with allergic disease, variations in affinity of specific IgE for natural allergen variants, such as homologous allergens from different grass pollen subfamilies, can affect the level of basophil activation.[25]

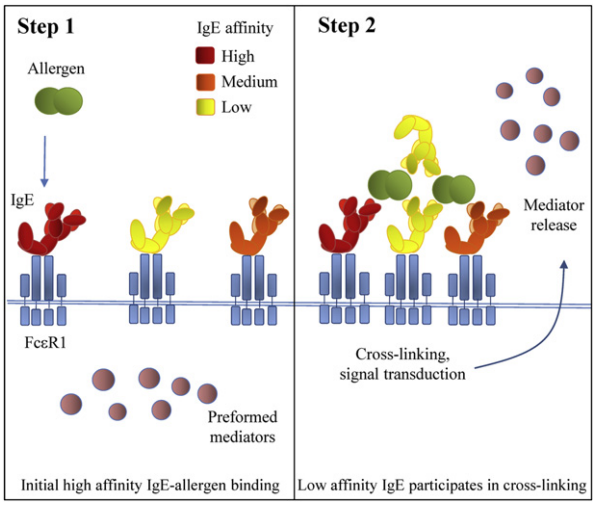

FIG 1. Low-affinity IgE contributes to basophil activation. FcεRI-bound IgE with moderate to high affinity for allergen takes the first step in binding to allergen. Subsequent binding of IgE to allergen *(Step 2)* does not require high-affinity IgE binding.[23] IgE of low affinity contributes to allergen cross-linking of FcεRI-bound IgE and activation of basophils or tissue mast cells in patients with allergic reactions.

POTENTIAL ROLE OF IgE IN EXPANDING THE IgE REPERTOIRE ACCORDING TO THE "INITIATOR ALLERGEN" HYPOTHESIS

The atopic IgE response to mites and pollen is unusual in at least 3 ways. First, the isotype-committed B memory component is almost absent. Second, the IgG/IgE ratio is generally less than 3 (ie, much lower than in the animal models),[26,27] which suggests that this atopic immune response might lack mature GCs altogether. Third, the repertoire involves many epitopes on many allergens. This is surprising because allergens are supposed to belong to a very special subset of antigens. If so, one would expect very few allergens to be present in an allergenic source material. In reality, at least 15 allergens have been described in the house dust mite *Dermatophagoides pteronyssinus*.

This unexpected complexity of the IgE response might be related to another role of IgE antibody in addition to its role in allergen-triggered activation of mast cells and basophils to release inflammatory mediators. The allergen-induced local reaction in the mucosa creates an environment that is conducive to the generation of additional IgE-switched B cells through mechanisms including T_H2 cytokines released from mast cells and basophils by using IgE-facilitated allergen presentation to T cells and recruitment of dendritic cells to sites of allergic inflammation.[28-32] In this way IgE can contribute to the amplification and perpetuation of additional IgE responses (ie, epitope spreading).[33] This involves the recruitment of newly generated IgE-switched B cells with new specificities to the same allergen or even to other allergens that happen to be present at the local site. Evidence indicating local class-switching to IgE has been found in mucosal sites of allergic subjects.[34,35] Statistical analyses of allergen array data obtained in longitudinal studies will be important to test the hypothesis that IgE antibodies with new specificities can be generated by bystander activation of IgE-committed B cells. Does the presence of IgE to Der p 1 increase the chance of the induction of IgE to Der p 2 more than the chance to induce IgE to a grass pollen allergen? Does induction of IgE to an indoor allergen, such as the cat allergen Fel d 1, in the presence of IgE to Der p 1 have a higher probability than the induction of IgE to an outdoor allergen, such as grass? Such an IgE-mediated epitope-spreading process suggests the possibility of an initiator allergen subsequently triggering an expanding IgE repertoire, as seen in longitudinal studies, as recently exemplified by Hatzler et al.[33] The "initiator allergen" hypothesis could help to explain the large number of allergens in most allergen source materials. If only the initial phase requires some special feature, only the initiator allergen would have to be a special type of antigen.

DIRECT AND INDIRECT SWITCHING PATHWAYS IN RELATION TO THERAPY

The comment by Xiong et al[22] that low-affinity IgE might be protective against allergic disease is presumably based on the idea that high levels of "irrelevant" IgE could saturate IgE receptors. This idea ignores the potent effect of IgE in increasing the number of IgE receptors on mast cells and basophils.[36] Attempts to use deliberate parasite infection have not supported the concept that low-affinity IgE is protective against allergy.[37] Although the anti-inflammatory activities of some parasites might be of therapeutic value, an increase in total IgE levels is not very effective.

IgE produced through the direct-switching pathway is, at the moment, an unattractive therapeutic target because it is produced by long-lived PCs without the involvement of a significant memory population.[1] Apart from interventions preventing the initial sensitization and homing, long-lived PCs resident in the bone

marrow are nonresponsive to allergen exposure or to most other immune-regulatory processes (including SIT). A possible exception is a therapy targeting the unique extracellular segment of the membrane form of IgE,[14,38-40] particularly in view of its increased expression on IgE-producing PCs.[14]

SIT is extensively used to alleviate allergen-induced symptoms. This treatment stimulates the development of IgG_4-switched B cells, which are well-established precursors for indirect switching to IgE in human subjects. Because the overall effect of the treatment is protective, this argues against the hypothesis that dangerous IgE is only produced through indirect switching. Paradoxically, both IgE and IgG_4 can be driven by the T_H2 cytokine IL-4, and both are associated with allergic disease.[41] However, IL-10 induced during SIT promotes B-cell synthesis of IgG_4.[42] We are yet to fully understand the balance between the role and ontogeny of IgG_4^+ B cells as precursors for IgE-switched B cells and IgG_4 as a subclass of immunoglobulin that blocks IgE and provides clinical benefits as a result of SIT.[43] A study of 7 paired IgE/IgG_4 transcripts from 4 patients with asthma has shown clonal relatedness of IgG_4 and IgE among 3 of these transcripts, which is consistent with indirect isotype switching to IgE.[44] Furthermore, both direct and sequential switching pathways to IgE have been observed in nasal tissue explants of patients with allergic rhinitis[45] and in *in vitro* cultured peripheral blood B cells.[46]

SUMMARY AND CONCLUSIONS

1. Some SHM and affinity maturation occurs even in the direct pathway of B-cell isotype switching to IgE.
2. IgE^+ B cells can be present in GCs but not in close proximity with T cells.
3. IgE-switched B cells show a predisposition for premature differentiation into PCs, and IgE^+ B memory cells are rare in the circulation.
4. The affinity requirements for IgE-mediated activation of mast cells and basophils by protein allergens are modest and likely to be met by IgE produced through the direct pathway.
5. Protective activity of low-affinity IgE in atopic allergy is unlikely because very high levels of IgE are needed to adequately saturate IgE receptors.
6. The main precursor in the indirect pathway, the IgG_4-switched allergen-specific B cell, is associated with tolerance rather than with allergen-induced symptoms.

The recent murine models for IgE responses are elegant and informative, but we are still left wondering about the cause and effect of the rapid exit of IgE-switched B cells (Fig 2).[1,6,11] The premature GC exit of IgE-switched B cells might reflect the unusual positive correlation between surface-expressed IgE (typical of B cells) and secreted IgE (typical of PCs) observed in the mouse model.[14] The surface expression needed for survival might require the activation of the PC program aimed at secreting large amounts of immunoglobulin. The murine models indicate that only very few IgE-switched B cells become IgE memory cells or manage to home as long-lived IgE^+ PCs in the bone marrow. This might fit with the observation of transient small IgE responses described in healthy young nonatopic children.[47] It would indicate that the availability of PC survival niches in the bone marrow during infancy is an important factor in the initial stages of the atopic march.

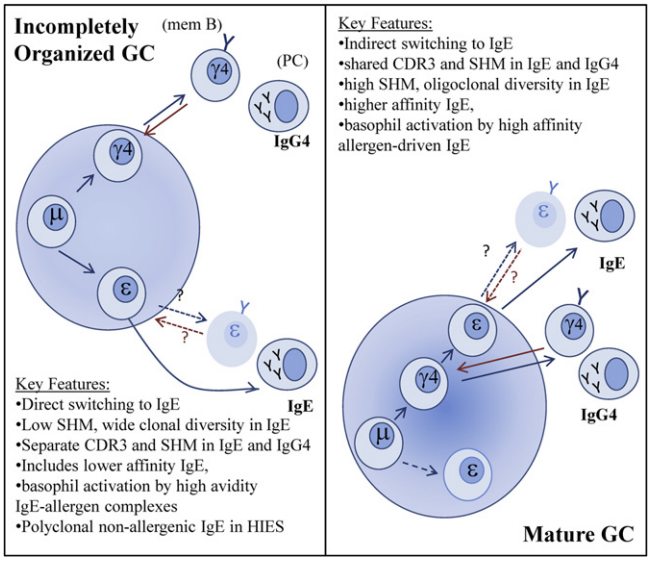

FIG 2. Models of development of IgE in human subjects. In an incompletely organized GC, such as might occur in the allergic type T_H2 milieu, B cells directly switch to IgE. IgE^+ switched B cells rapidly exit the GC and differentiate into PCs, which is incapable of expansion and further GC activity.[11] Existence of circulating memory B *(mem B)* IgE^+ cells is contentious *(faded shading)*. IgE^+ B cells have low surface expression of IgE[6] and do not compete within a mature GC for antigen and survival signals from T follicular helper cells (IL-21). In a mature GC, B-cell precursors indirectly switch to IgE through allergen-specific, somatically mutated IgG_4 memory B cells, giving rise to high-affinity allergen-driven IgE. Rare IgE^+ memory B cells might exist in human subjects to provide a pool of B cells to sustain IgE memory through direct differentiation to PCs. The figure was adapted and developed from Aalberse and Platts-Mills.[1]

The extent of GC involvement in allergen-driven B-cell activation, isotype switching to IgE, SHM, and affinity selection for allergen remains to be fully clarified. The higher cross-reactivity of IgE antibodies compared with IgG_4 antibodies might reflect a less stringent antigen-dependent selection caused by the short residence time in the GC in patients with allergy.[48] Further research into the pathways of B-cell switching to IgE and the mechanisms of IgE memory in patients with allergic conditions is essential to establish a solid knowledge basis from which new approaches to the management of these diseases can be developed.

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