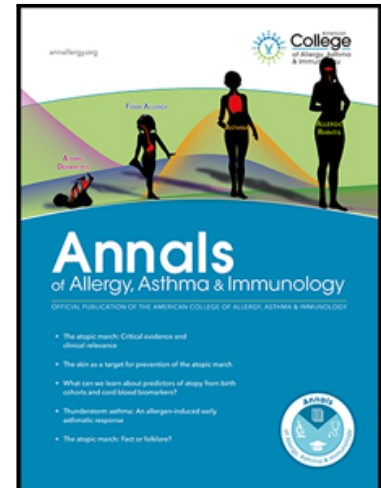


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Do germinal centers protect most of us from becoming allergic?

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Title: Do germinal centers protect most of us from becoming allergic?

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Abbreviations/Acronyms:

GC: germinal Center

sIgE: allergen-specific IgE

sIgG: allergen-specific IgG

Abstract

Objective: To review the literature and discuss a hypothesis as to why most people do not become allergic. This hypothesis is dependent upon three main components. (1) Airborne allergens (for example from pollen or mites) are weak antigens that induce a B-cell response only in immunologically most reactive subjects (i.e., atopic). (2) A roadblock to production of IgE is the TH2/IL-4 requirement for class switch to IgE. (3) Activated germinal centers prevent the formation of mature IgE-switched B-cells, creating a second roadblock to IgE production.

Data Sources: Transgenic reporter mice and a cross-sectional human cohort.

Study Selections : From the mouse studies we selected the data on histology and tissue-derived cell suspensions published by several groups in 2011-2014. From the human cohort we selected our published micro-array data on the levels of allergenspecific IgE and IgG in serum.

Results: The immune response to airborne atopic allergens entails both IgE and IgG antibodies rather than just an IgG or IgE response. However, as expected for an immune response without mature germinal centers, the specific IgG levels will be very low, typically in the ng/ml range.

Conclusion: Control of IgE production is not just through TH2/IL-4 mediated class switch. Recent studies suggest that mature germinal centers are likely to provide protection against the development of allergy to airborne allergens, as well. This may explain why allergen exposure does not induce allergen-specific IgE in everyone.

Key messages:

- IgE-switched B cells differ strikingly from IgG-switched B cells.
- The class-switch to IgE is not the only roadblock on the way to IgE-mediated allergy.
- Immunization that induces mature germinal centers is unlikely to result in allergic sensitization via direct switching from IgM to IgE, but may do so via an indirect switching pathway, from IgM via IgG to IgE.
- The IgG/IgE ratio to allergens from mites and pollen is usually <10 In atopic patients, which is much lower than the IgG/IgE ratio in rodent model systems and in human non-atopic sensitization e.g. to insect venom.
- Due to the competitive inhibition by the excess of IgG with the same specificity, IgE produced via the indirect switching pathway is less likely to trigger an allergic reaction.
- Our findings support our hypothesis that mature germinal centers are likely to provide a protection against the development of allergy to airborne allergens.

Introduction

Not everyone who is exposed to an allergen makes IgE antibodies to the allergen. One of the crucial questions concerning allergy is: what prevents most of us from producing IgE antibodies to allergens? In this paper we restrict our discussion to classical airborne allergens from pollen and mites, focusing on the formation of allergen-specific IgE, but not discussing the immunological and clinical consequences of subsequent allergen exposure. We provide a framework to consider on the route from allergen exposure to development of IgE mediated allergy. This novel framework provides a potential explanation for one of the most vexing questions in IgE biology and allergic disease -- why is it that allergen exposure does not induce allergen-specific IgE in everyone?

From naïve B cells to IgE-producing plasma cells

The subject of this paper is the developmental path of a naïve B cell to an IgE-producing plasma cell. This pathway occurs in the early phase of allergic sensitization, which typically occurs in draining lymph nodes. Initially, B cells make IgM antibodies. Upon antigenic stimulation the B cell may switch to another class, for example from IgM to IgG or IgE. This class switch is an irreversible process. The B cells may further differentiate and proliferate in germinal centers (GCs) within the lymph nodes. The most important outcomes for antigenic stimulation of B cells are cell death or either differentiation to a memory B cell or into a terminally differentiated plasma cell. The antibodies that are excreted into the extracellular space and circulate throughout the body are largely produced by plasma cells.

Below we discuss two impediments to development of an IgE-switched B cell and subsequently an IgE-secreting plasma cell. The first obstacle is class switching to IgE, and the second is survival of the IgE-switched B cell, particularly in a mature GC.

The first obstacle on the road to IgE-producing cells

The first roadblock, class switching to IgE, results from a dependence on sufficient TH2 help. This dependency is not absolute, because some IgE B cells can be found in patients with T cell deficiencies. However, in relation to allergy, the need for TH2 help is undisputed. One obvious assumption is that insufficient TH2 help is the most important reason for a lack of allergen-specific IgE (sIgE) in most of us. Supporting this argument for TH2 help is the evidence that risk of allergic sensitization is decreased with a history of exposure to some microbial factors. Indeed, this is the basis of the hygiene hypothesis: microbial factors inhibit the development of TH2 cells and thus inhibit the IgE switch¹. This protection by microbial factors is transient, as the microbial factors need to be present at the time of allergen exposure². It is unlikely that the presence or absence of TH2 help is the only obstacle on the route to allergy. Indeed, if the lack of TH2 help were the only roadblock to the development of IgE B cells, it would be difficult to explain why allergic children do not become IgE sensitized to all allergens in their environment. Another argument against a singular requirement for TH2 help relates to the polyclonality of the allergen-specific IgE response. If the IgE-switch was entirely dependent upon only TH2 cell help, then after this difficult switching event we would expect expansion of the IgE-switched B cells to be similar to clonal expansion of IgG-switched B cells. Thus, this expansion often would result in a monoclonal IgE-B cell population that originated from a single

IgE-switched B cell. However, analyses of sIgE binding from allergic subjects to allergen-derived peptides indicate that the IgE response is actually polyclonal (from multiple B cells)³.

This observation fits with the requirement for allergen-induced mast cell triggering. An allergen needs to cross-link at least two cell-bound IgE molecules to trigger a mast cell. Most allergens are monomeric and, thus, will be unable to crosslink two IgE antibodies that have the same epitope specificity. Since each B cell produces just one type of IgE antibody, a single B cell only can produce an IgE that recognizes a single epitope. This implies that for any clinically relevant allergen several IgM B cells must undergo class switch to IgE; thus, the switch to IgE must not be an extremely rare occurrence.

The second obstacle: a GC eliminates IgE-switched B cells.

IgE-switched B cells are rare compared to IgG-switched B cells. This may indicate these two types of B cells differ in more than just the class of antibody they produce. Could a post-switch effect contribute to the rarity of IgE production? Our working hypothesis is that one or more GC-related effects do play an important role in limiting the amount of IgE producing cells.

A GC is a conglomeration of B and T cells that forms in a lymph node or the spleen upon antigen exposure and subsequent antigenic stimulation. Development from an immature to a mature GC takes several days. A mature GC refers to the explosive proliferation of B cells that occurs upon antigenic stimulation. At the same time the B cells proliferate, the DNA encoding their antibodies is subject to somatic hypermutation, with mutations occurring mostly in the gene segments coding for the antigen-binding regions. These mutated B cells are positively selected based on their reactivity with the antigen that is still being presented in the lymph node follicle.

B cells that have inferior antigen reactivity die by apoptosis. Following this clonal expansion, surviving B cells may differentiate into memory cells or terminally differentiated plasma cells.

The observation that IgE-switched cells produce only few daughter cells as compared to IgG-switched cells strongly suggests that IgE-switched cells do not take part in the GC reaction. Further evidence that IgE switched cells are not part of the GC reaction comes from studies demonstrating the scarcity of IgE memory B cells in humans and mice⁴. Indeed, this provides a potential explanation for the observation that injection of allergen during immunotherapy results in a much less pronounced increase in sIgE compared to allergen-specific IgG (sIgG) (2-5 fold versus 10-100 fold)^{5,6}. In 2004, based on these observations, we postulated that IgE-B-cells are eliminated upon maturation of GCs⁷. This hypothesis was based on indirect evidence at the time. In 2012-2013 three laboratories reported that upon primary immunization the development of IgE- and IgG-B cells had different fates⁸⁻¹⁰. These studies used transgenic reporter mice and a very sensitive and specific procedure to locate switched B cells. B cells that had switched to IgE also would produce a fluorescent protein, allowing their identification immediately after the class switch. Using these mice, the investigators found that up to the end of the first week post immunization, IgE-switched cells developed identically to IgG-switched cells. However, once the GC started to function and IgG-switched cells began explosive proliferation, the number of IgE-switched B cells rapidly declined¹¹⁻¹³.

What is so special about the early phase of atopic sensitization?

A controlling role of GCs as described above explains (in combination with the TH2/IL-4 dependency) why IgE antibodies are produced in only minimal quantities most of the time. It is important to note that this does not explain why some people develop allergies while others

remain asymptomatic. One possible clue is that formation of GCs is stimulated by danger signals of microbial origin⁴. Therefore, upon contact with an antigen in the absence of such danger signals the B cell signal will be so weak that only the most reactive people will react -- and even then this doesn't always happen. Otherwise if everyone reacted, we would expect atopic children in the same household to have the same allergic sensitivities, but we do not see that; indeed, it is not uncommon to see one child with hay fever, another with mite allergy, and a third with cat allergy all in the same household.

In figure 1 we show two stages of our working hypothesis: 1) At the primary phase of allergic sensitization exposure to classical allergens (e.g. from pollen or mites) results in only weak antigenic stimulation; 2) the atopic constitution is characterized by a relatively strong B cell response to weak antigenic stimulation. As shown in the figure, it is generally accepted that the IgE response depends on T cell help, which in turn was dependent on the help from several other cells, such as dendritic cells. However, the two other aspects of our hypothesis are not yet widely accepted and are discussed in more detail below.

Working hypothesis part 1: atopic allergens are weak antigens

A popular opinion is that allergens are exceptional proteins. This thesis has been defended for many years by Breiteneder and coworkers¹⁵ who extensively investigated the pedigrees of allergens and non-allergens based on evolutionary and structural information. One of their arguments is that allergens belong to a small, exclusive group of protein families and that most protein families do not contain allergens. However, defining non-allergens by exclusion (i.e., lack of evidence for the protein to cause allergies) is problematic and not satisfactory. Therefore, in order to conclude a protein is not an allergen, we must first verify that the immune system of

atopic subjects is being adequately exposed to the protein. In terms of airborne allergens this means the protein has to be water-soluble and not too big or small (50-500 amino acids)¹⁶. For the allergen to be inhaled, a carrier particle is needed, because free floating allergens are inhaled in only exceptional situations. Pollen and other dusty particles in principle are suitable carriers, but need to fulfill other requirements, such as being able to carry a large amount of the allergenic protein, rapidly release the allergenic proteins when in an aqueous medium and not induce TH2-suppressive activity. Allergenic proteins (and carriers) that fulfill these requirements are far from rare, suggesting that their only special feature is inefficiency in stimulating the immune system¹⁷.

Working hypothesis part 2: an atopic constitution is characterized by a low threshold for B cell activation.

Our working hypothesis assumes that most of the time allergens are “invisible” to the immune system, but there may be an occasional situation in which the stimulus is just strong enough to elicit a reaction in B cells. We propose that this happens more often in subjects with a genetically more reactive immune system¹⁸, although even in this subpopulation exposure to an allergen will often pass unnoticed. If the B cells react to the stimulus, a weak response will be induced that is inclined to fizzle out before fully active GCs develop. In the absence of active GCs, both IgG-switched as well as IgE-switched B cells will leave the follicle. The clonal expansion will be modest and both IgG and IgE B cell numbers will be small. Also the level of somatic hypermutation will be modest and few if any memory B cells will be generated. The few antigen specific B cells might migrate to the bone marrow and differentiate into IgG- or IgE-producing plasma cells¹⁹. It has been suggested that in the absence of GCs, affinity maturation is modest and might be insufficient for IgE to activate mast cells upon allergen contact. However, the

affinity requirement for IgE-allergen activation of mast cells is lower compared to antibodies that need to neutralize pathogens or toxins²⁰. In reality, the relevant parameter in IgE mediated activation is the dissociation rate. Mast cell activation takes less than fifteen minutes, so there is no need for IgE to keep hold of the allergen longer. In contrast, pathogens and toxins may need to remain antibody-bound for many hours.

Are the results of mouse experiments applicable to atopic humans?

We still don't have adequate mouse models for atopic allergies, even if we only restrict our discussion to IgE antibody production and leave out symptomatic disease. An important shortcoming of almost all experimental models is that the protocols used result in a close to 100% chance of IgE production⁸⁻¹⁰. One essential aspect of allergy is that it is an exceptional situation. Thus, it would be more informative to have a number of protocols in which only 20-40% of inbred animals produced sIgE and then look for differences between the responders and non-responders.

One of the differences between the antibody response in atopic subjects and mouse models is the IgG/IgE ratio. Unlike in the human, in most mouse models the sIgG levels are much higher than those seen in untreated atopic patients. These high sIgG levels in experimental animals (often more than 10,000 ng/ml) point to an immune response with active GC^{11,18}. This is markedly different from what has been seen in humans in regards to specific IgG (sIgG) and sIgE levels to airborne allergens.

The sIgG response to an airborne atopic allergen in human subjects with and without an IgE response to the same allergen differs markedly. In IgE-positive subjects the sIgG response is in the range of 30-300 ng/ml, whereas in IgE-negative subjects the sIgG response is usually less

than 10 ng/ml. This might suggest the use of sIgG as a diagnostic test instead of sIgE, but for technical reasons (high non-specific IgG background signals) the sIgG test is much less reliable in this low antibody range than the sIgE assay. A schematic overview of situations with distinct IgG/IgE ratios is shown in figure 2 (based on data from²¹).

Often there is an assumption that an immune response consists of either IgG or IgE, and that the induction of sIgE indicates that the inducing antigen is an “atopic” allergen, but this is just not the case. In many immune responses both IgG and IgE are produced, and indeed we propose to use the ratio of IgG/IgE to a specific allergen to classify the allergen as atopic or not. Allergens that induce upon natural exposure usually induce an IgE antibody response with an IgG/IgE ratio of <10 can be considered atopic (most pollens and mites), while those with a ratio of ≥ 10 are considered non-atopic. These non-atopic allergens include insect venom, airborne occupational allergens, and food allergens (like milk and egg), and in some (but not most) individuals can still cause allergic disease. Some vaccines, like tetanus and diphtheria toxoids (if void of cellular pertussis), generate robust IgE responses along with IgG; the very high IgG/IgE ratio, however, indicates these are not atopic allergens. As with venom, occupational, and food allergens, most individuals will have no allergic symptoms when exposed to an allergen that is not atopic.

The second phase of allergic sensitization: expansion and diversification

From the immunological point of view, the situation of IgE production upon secondary allergen exposure in a previously sensitized subject differs from the textbook secondary IgG immune response. Specifically, IgE-switched classical memory cells are virtually absent, because they are generated in mature GCs. Yet, a variant secondary IgE response is found, as best described in

publications from the Gould lab²². Despite the lack of IgE memory cells, secondary IgE B cells do appear, and have been found to be descendants of IgG memory cells. In order for these IgE B cells to have descended from IgG memory cells, a second class-switch is required--first a class-switch from IgM to IgG, followed by a switch from IgG to IgE. In the case of airborne allergens this formation of new sIgE-producing B cells from sIgG memory cells is observed mostly in the mucosa of allergen-exposed airways. At this location, tertiary lymphoid structures develop during allergen-induced type-2 inflammation. This process is markedly influenced by many types of cells, such as eosinophils, basophils and specific T cell subsets (TH2- and Tfh-13 cells)²³, as well as by various cytokines, such as the IL-4-related cytokine IL-13, and the IgE-regulatory cytokine IL-21^{24,25}. Following the switch from IgG to IgE, the B cell can differentiate into an IgE-producing plasma cell. These plasma cells may be able to survive in the inflamed mucosa and produce sIgE antibodies for several months. In the case of seasonal allergens this results in a marked post-seasonal increase in sIgE^{11,19}. This increase can be prevented by interventions that dampen allergen-induced mucosal inflammation, such as classical allergen-specific immunotherapy.

The primary relevance of this secondary sIgE response is not the increase in sIgE, because these secondary IgE B cells have the same epitope specificity as their sIgG B cell parent. This clonal relationship between large numbers of sIgG-producing B cells and a few secondarily-switched sIgE-producing B cells is predicted to generate an excess of IgG antibodies that are optimally suited to compete for the allergen with the IgE antibodies. A much more relevant aspect is the diversification of the spectrum of the IgE antibodies in tertiary lymphoid tissue²⁵. This diversification may occur at three levels. We will use grass pollen as example. If we assume that the initial IgE-response is directed against the grass pollen allergen Lol p 1, then this

secondary IgE response can facilitate the formation of bystander allergens such as 1) other epitopes of the Lol p 1 protein; 2) epitopes on other allergens from grass pollen; 3) epitopes on allergens of other sources, such as weed pollen that happens to be inhaled simultaneously with the grass pollen. Where this diversification occurs is still controversial, but it would be expected to occur outside of traditional GC, as discussed above.

Conclusion

Regulation of IgE is complex and likely relates to why not everyone who makes sIgE has allergies. In this review we have discussed potential reasons for how and why the IgE-producing B cell regulation may matter. IgE-switched B cells differ strikingly from IgG-switched B cells. In addition to the requirement for TH2 help, IgE producing B cells need to avoid the development of mature GC. This is why immunization that induces mature germinal centers is unlikely to result in allergic sensitization via direct switching from IgM to IgE, however, it is still possible for allergic sensitization to develop via an indirect switching pathway, from IgM via IgG to IgE. We also discussed the concept that the IgG/IgE ratio to allergens from mites and pollen is usually <10 in atopic patients, a ratio which is much lower than that seen in rodent model systems and in human non-atopic sensitization (e.g. tetanus vaccination^{27,28}). Taken together our findings and those from other groups supports the hypothesis that mature GC provide a protection against the development of allergy to airborne allergens (atopic allergens). This review provides a framework for future research into the production and blockade of IgE, as well as why allergies are not universal in the population.

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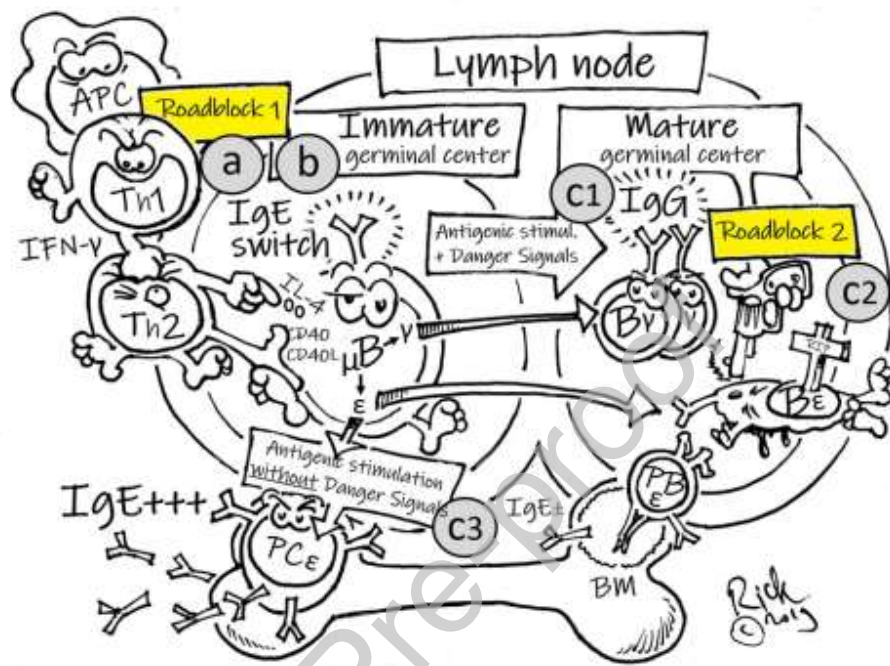


Figure 1. **Working hypothesis. Two roadblocks on the way to atopic sensitization.** (a)

Roadblock 1: In contrast to IgG production, the production of allergen-specific IgE (sIgE) needs TH2 cells. Upon antigen contact, microbial factors ('danger signals') and interferon-gamma producing TH1 cells suppress the development of TH2 cells. (b) In the absence of Th1 suppression, TH2 cells can activate naïve B cells located in the B cell follicles via protein secretion (IL-4 and IL-13) and stimulating cell-to-cell contact (CD40 ligand on the T cell and CD40 on the B cell). (c1-3) With the right strength of stimulation, germinal centers form in the follicles and B cells are induced to switch from IgM to production of other Ig classes, such as IgG and IgE. (c1) In mature germinal centers, IgG-B cells are further stimulated (clonal expansion, development of B memory cells). (c2) Roadblock 2: The fate of IgE-B cells are very different -- the majority of the IgE-switched B cells are eliminated, although some will escape

from the germinal center as plasmablasts (PB). (c3) An atopy-specific feature: In the absence of strong antigenic stimulation and danger signals, immature germinal centers simmer awhile and then fizzle out. In this situation, clonal expansion is minimal for both IgE- and IgG-B cells. Only a small number of antibody-secreting plasma cells (PCs) will form and might find a survival niche in the bone marrow, but this may still result in sufficient IgE to cause allergic symptoms.

APC = dendritic cells; BM = bone marrow (modified from reference 14).

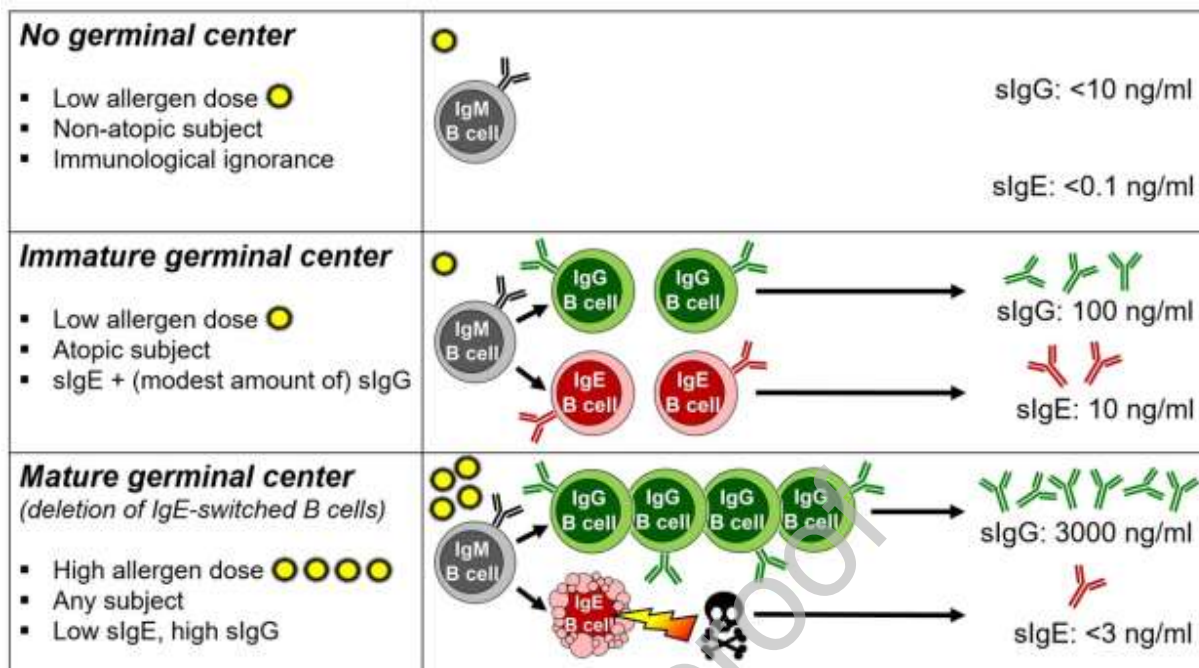


Figure 2. **Relationship of IgG and IgE with antigenic stimuli.** The upper row indicates the situation in the case of a weak antigenic stimulus in a non-atopic person: The immune system does not react. The middle row indicates the situation that is most likely to result in symptomatic allergy, in the case of a weak antigenic stimulus, in an atopic person: the immune system does usually react, but the stimulus induces at most an incomplete response without germinal centers, which results in IgE B-cells that are not removed, but only little IgG -- indeed almost as little IgG as IgE. The lower row indicates the situation with a strong antigenic stimulus: the immune system reacts with generation of mature germinal centers that stimulate the proliferation of IgG-producing B-cells, whereas IgE-producing B-cells are eliminated.