

How do we avoid developing allergy: Modifications of the T_H2 response from a B-cell perspective

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The synthesis of IgE by B cells occurs at a low rate compared with that of other antibodies, even in allergic subjects. One rate-limiting step is the class switch, by which B lymphocytes switch to produce immunoglobulin ϵ heavy chains rather than μ or γ heavy chains. We propose an additional rate-limiting step: survival of the B lymphocyte after the switch to IgE. The hypothesis we present here is that the survival of the IgE-switched B cell is compromised, particularly in an active germinal center. Antigenic stimulation in the absence of a danger signal fails to induce a mature germinal center, which allows IgE-switched B cells to escape and mature into plasma cells. Antigenic stimulation in the presence of a danger signal (or under nonhygienic conditions) induces germinal centers, which eliminate IgE-switched B cells. Thus the essence of an allergen is antigenic stimulation in the absence of conditions that generate mature germinal centers. Because germinal centers are important for the generation of B-cell memory, the IgE immune response is characteristically poor in memory (but might be long lasting because of the generation of long-lived plasma cells). In addition to this direct route to IgE, typical for atopic sensitization, another type of T_H2 response exists. On chronic allergen exposure with the concomitant induction of germinal centers, IgG4-switched B memory cells are induced that are slow to differentiate into plasma cells. These IgG4-switched B memory cells might occasionally undergo a secondary switch to IgE. (*J Allergy Clin Immunol* 2004;113:983-6.)

Key words: *IgE regulation, IgG4, T_H2, germinal center, class switch, hygiene hypothesis, atopy*

THE B CELL BETWEEN HYGIENE AND DANGER

The primary role of the immune system is to protect us from infections, and a central element of this is the ability

to recognize foreign antigens. In modern society, but not in traditional rural societies, up to 30% of the population responds inappropriately to otherwise benign foreign proteins, such as pollens, dust mites, or mold. Exposure to these proteins, which are generally inhaled and only in nanogram quantities per day, gives rise to IgE antibodies. Although low in absolute quantities (ie, $\leq 0.01\%$ of serum immunoglobulin), these antibodies can persist for years and give rise to severe allergic symptoms. Increasing evidence suggests that the initial recognition of foreign proteins is in many cases triggered through receptors of the innate immune system, which recognize danger signals. In keeping with this, Matzinger¹ suggested that the allergens are distinguished because they signal danger to the immune system. On the other hand, the classical view on the cleanliness hypothesis argues that high exposure to endotoxin or other microbe-derived factors can steer the immune system away from IgE antibody production by favoring the production of T cells of the T_H1 type. (For alternative views on how cleanliness and pathogens might change the workings of the immune system, see Bach,² Yazdanbakhsh et al,³ and Van der Kleij and Yazdanbakhsh.⁴) Production of IgE antibodies is dependent on the T_H2-derived cytokine IL-4. It is important to realize that IgE antibodies are not a significant fraction of the response to (1) bacterial or viral infections, (2) most immunization schedules in human subjects, and (3) any immunization regimen in mice that includes high-dose antigen or CFA.

It is well recognized that conditions that induce a T_H1 response induce germinal centers and are in general highly suppressive to T_H2 responses, including IgE antibody production. On the other hand, it has become clear that exposure to some allergens, such as cat, bee venom, and rat urine, can induce antibody responses that include the IL-4-dependent isotype IgG4 but often do not concomitantly induce IgE antibody.⁵ In recent studies the role of T cells in controlling IgE antibody has been regarded as paramount, with very little consideration of the nature of the B-cell responses. In this article we will argue that mature germinal centers are the normal control system that prevents IgE antibody responses to most antigens, and

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persistent IgE antibody responses occur primarily with an antigenic stimulus that is subliminal in the sense that it is not sufficient to induce germinal centers. The main elements of this argument come from (1) murine evidence that memory cells switched to the IgE isotype do not survive in the germinal center, (2) a wide range of human evidence that IgE antibody responses have a poor or absent B memory component, and (3) the recent evidence that T_H2 responses can be modified to a form in which IgE antibodies are not produced. In this model the crucial feature of allergens is that they lack the signals to induce germinal centers, either because of low doses or because of a lack of danger signals.

HOW ARE IgE ANTIBODY RESPONSES CONTROLLED OR PREVENTED: CLASS SWITCH TO ϵ IS NOT THE ONLY LIMITING FACTOR

Why don't we make more IgE antibodies (and more often)? Even when the short half-life of IgE is taken into account, it is obvious that IgE synthesis occurs at a lower rate than that of other immunoglobulin isotypes, such as IgG1, IgG4, or IgA. This is true even in allergic subjects. What are the rate-limiting steps that distinguish IgE production from IgG production? A critical step is undoubtedly the class switch. This is a complex scheme of events leading to the DNA rearrangements that are required for the synthesis of an immunoglobulin heavy chain different from the μ heavy chain. (For a recent review, see Geha et al.⁶) In the case of IgE antibodies, heavy chain production in the B cell has to switch, either directly or sequentially, from μ to ϵ . The availability of adequately activated T_H2 cells is essential to induce the B cell to switch. This activity of T_H2 cells is susceptible to downregulation by many of the classic danger signals. However, it is very unlikely that the class switch is the only element that controls IgE antibody production. First, IgE antibody responses are polyclonal in the sense that many different B-cell clones are involved,^{7,8} implying that IgE switch has occurred repeatedly. Second, the clonal size of IgE-switched B cells is much smaller than that for other antibodies,⁹ and IgE-switched B cells are strikingly susceptible to apoptosis induced by cell crowding.¹⁰ Thus there must be mechanisms controlling the expansion of IgE B cells after the switch to the expression of the gene for IgE heavy chains.

SURVIVAL OF IgE-SWITCHED B CELLS IS COMPROMISED

Much of what we know about IgE production in vivo and in vitro supports the hypothesis that the second control, which operates on B cells after the class switch to ϵ , is linked to B-cell survival. At least 2 mechanisms could selectively limit the survival of ϵ -switched cells. Within the germinal centers survival is dependent on survival signals that protect B cells from apoptosis. There is

evidence that the membrane form of the ϵ chain is defective.¹¹⁻¹⁵ This defect prevents rescue of IgE B cells because the major survival signals act through the B-cell receptors. The other is an interaction between membrane-anchored IgE on the B cell and the low-affinity receptor for IgE, CD23, which is present on a cell that is heavily involved in B-cell selection, the follicular dendritic cell.¹⁶ Studies on the anatomy of the humoral immune response in mice suggest that the germinal centers are involved in the negative selection of ϵ -switched B cells. Germinal centers are induced in the B-cell area of secondary lymphoid tissue after a strong immune stimulus. Within germinal centers, B cells proliferate and differentiate. This requires strict quality control through negative and positive selection. B cells that do not receive survival signals undergo apoptosis. One of the proteins that transmits survival signals is Bcl-6. In mice genetically deficient in Bcl-6, mature germinal centers are not formed; however, IgE responses are unaffected.¹⁷ Similarly, in the absence of cathepsin S, the formation of germinal centers is disturbed, as is the switch to IgG2a and IgG3, but IgE production is not affected.¹⁸ These studies indicate that in contrast to IgG, IgE production does not depend on mature germinal centers. However, the induction of B-cell memory requires functional germinal centers. This fits with the notion that conditions that favor memory induction are unfavorable for IgE production.

IS SEQUENTIAL SWITCH OF HEAVY CHAIN GENES THE PRIMARY ROUTE TO IgE?

There is good evidence that the production of IgE can occur through sequential switching events from μ to γ^4 to ϵ .¹⁹ (In mice the intermediate is IgG1.) In this model the production of IgG4 without IgE antibody would be seen as a restricted or truncated T_H2 response. In this communication we propose that the modified T_H2 response results from a failure of the direct switch mechanisms combined with increased generation of IgG4 memory.

In the animal models it appears that weak antigenic stimulation (ie, low dose, without microbial adjuvant factors, or both) favors the development of IgE plasma cells through direct switch from μ to ϵ .²⁰ This mechanism occurs without much clonal expansion or the formation of a B memory population. Further evidence suggests that these responses can give rise to a long-lived plasma cell population without effective production of B memory cells. Stronger stimulation results in memory cells switched to IgG/IgG4, which potentially can switch to IgE. However, because of the problems outlined above, the survival of IgE memory cells is severely compromised (Fig 1). In mice long-lived IgE antibody responses like those in human subjects can be induced, but they are also relatively insensitive to subsequent antigenic stimulation.²¹⁻²³ We suggest that these responses in both mice and human subjects represent long-lived plasma cells without B memory.

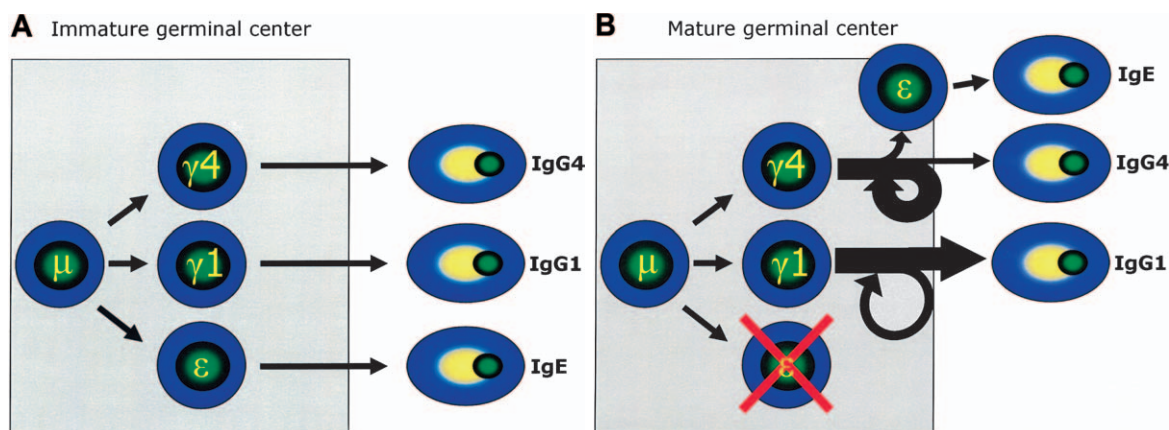


FIG 1. A, Immature germinal center. A weak T_H2 response fails to induce a mature germinal center. The resulting antibody response is low and nonresponsive to subsequent antigen exposure (because of a lack of B memory cells). **B, Mature germinal center.** A strong T_H2 response induces a mature germinal center. ϵ -Switched B cells are largely eliminated. Some IgE production might occur through γ 4-switched B cells. On subsequent antigen contact, γ 1 memory cells largely differentiate into plasma cells. In contrast, γ 4-switched memory cells mostly proliferate to generate more memory cells, with only a few cells differentiating into plasma cells. Round cells are B lymphocytes, whereas oval cells are immunoglobulin-secreting plasma cells. Circular arrows indicate the expansion of B memory cells.

The extent of B memory in human subjects can be judged either from the postseasonal increase in IgE antibody in patients with pollinosis or the response to allergen-specific immunotherapy. These responses are characteristically slow and small. There is certainly a striking contrast between the robust IgG antibody responses and the IgG4 antibody responses to immunotherapy and the modest increase in IgE antibody.

ATOPIC AND NONATOPIC T_H2 RESPONSES

When combining these data on human and rodent antibody responses in vivo with cellular and subcellular phenomena, the following picture emerges (Fig 1). A weak T_H2 response (eg, exposure to pollen or mites) fails to induce a mature germinal center. In this situation activated B cells undergo some somatic mutation and an isotype switch but do not develop into memory cells. Through lack of Bcl-6, the differentiation protein Blimp-1 is expressed, and B cells differentiate into plasmablasts. Some of these reach the bone marrow and become established as long-lived plasma cells. Because these processes are inefficient, the resulting antibody response is low and nonresponsive to subsequent antigen exposure (because of lack of B memory cells). A strong T_H2 response (eg, high exposure to cat allergen or bee venom) induces a mature germinal center. In this environment activated B cells can only survive if they are stimulated through their surface immunoglobulin. ϵ -Switched B cells are eliminated because their membrane immunoglobulin is defective, because they receive a negative signal through the low-affinity IgE receptor (CD23) on the follicular dendritic cell, or both. Some IgE production might occur through γ 4-switched B cells (indirect switching). On subsequent antigen contact, γ 1 memory

cells largely differentiate into plasma cells. In contrast, γ 4-switched memory cells mostly proliferate to generate more memory cells, with only a few cells differentiating into plasma cells.

IgE production is inefficient because ϵ -switched B cells are eliminated during the normal germinal center reaction. The IgE antibodies that might be found results from either of 2 unconventional routes: (1) a direct μ -to- ϵ switch during the early phase of the germinal center reaction, in which B cells escape from the lymphoid follicle and become established as a long-lived plasma cell in the bone marrow, or (2) indirect isotype switching, in which IgE-producing plasma cells are derived from γ 4-switched memory B cells. The first process reflects classical atopic allergy (ie, IgE formation after low-dose exposure to airborne allergens from pollen and mites). In this allergic type of immune response, there is only a minor B-cell memory compartment but a persistent IgE antibody response caused by long-lived plasma cells. The alternative process is induced on high-dose exposure to allergens. This results in a T_H2 response with a strong memory compartment (predominantly IgG4). The choice between the 2 routes depends on the extent and the speed of the establishment of a well-organized germinal center that will eliminate the majority of ϵ -switched B cells.

CONCLUSIONS

The increase in the prevalence of IgE-mediated allergy has been linked to a lack of infections, particularly during early childhood. This has been postulated to reflect a lack of a microbial-induced shift in the T_H1/T_H2 balance.²⁴ However, microbes might prevent allergic sensitization in 2 additional ways: (1) by inducing the maturation of germinal centers and (2) by decreasing the number of

survival niches for plasma cells.²³ Early B cells appear to compete with plasma cells for the same survival niches in the bone marrow. An increase in the number of B cells produced in the perinatal period might result in a decrease in the number of bone marrow plasma cells.²⁵

The recent evidence that high exposure to cat and some other allergens can produce a tolerant immune response including IgG4 antibody without IgE antibody has led to a new appreciation of the complexity of B-cell development. A major part of our argument is that mature germinal centers are an important site for the control of IgE antibody responses. Thus the increase in IgG4 antibodies without IgE antibody most likely reflects increased production of allergen-specific B memory cells in an environment that does not support the expansion of IgE B cells. In this model an allergen is a foreign protein delivered in low dose or with inherent biologic properties that lack the danger signals necessary to induce a T_H1 response or to generate mature germinal centers. The modified T_H2 response is modified in 2 ways: the direct switch to IgE plasma cells is restricted, and the generation of B memory cells for IgG4 is enhanced.

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