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Targeting IgE production in mice and humans

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Immunoglobulin E (IgE) is pathogenic in allergic diseases such as asthma, allergic rhinitis, atopic dermatitis, and food allergy. Recent studies using genetically modified IgE reporter mice indicate that the majority of serum IgE in mice is produced by short-lived IgE plasma cells, with minor contributions from long-lived IgE plasma cells, and implicate IgG1 and IgE memory B cells as potential sources of IgE memory. Clinical studies using antibodies against IL-13 or the IL-4 and IL-13 receptor subunit IL-4Rα, as well as an antibody against the M1 prime domain of human membrane IgE, indicate that, similar to mice, a proportion of IgE in humans is derived from ongoing IgE immune responses and short-lived plasma cells. Targeting IgE production may lead to new therapies for the treatment of allergic diseases.

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Introduction

Immunoglobulin E (IgE) mediates anaphylaxis reactions that are pathogenic in allergic diseases such as asthma, allergic rhinitis, atopic dermatitis, and food allergy [1]. In patients with these diseases, total and allergen-specific IgE levels are elevated compared to healthy individuals. Treatment of moderate-to-severe asthmatics who are poorly controlled on inhaled corticosteroid therapy with a neutralizing anti-IgE monoclonal antibody (omalizumab) decreases free serum IgE levels and reduces asthma exacerbations [2]. Omalizumab does not significantly affect IgE production in these patients, at least in the first year of treatment [3]. Therefore, therapies that inhibit IgE production may yield new treatments for allergic diseases. In this review, we summarize our understanding of IgE production *in vivo*, focusing on recent studies in mice that examine the biology of IgE-producing plasma cells and the sources of IgE memory. We discuss approaches for inhibiting IgE production either by neutralizing the cytokines IL-4 and IL-13 or by targeting IgE-switched B cells directly through the membrane IgE B cell receptor (BCR). Finally, we summarize the effects of therapeutics targeting IL-4, IL-13, IL-4Rα, or the membrane IgE BCR on IgE production in human clinical studies.

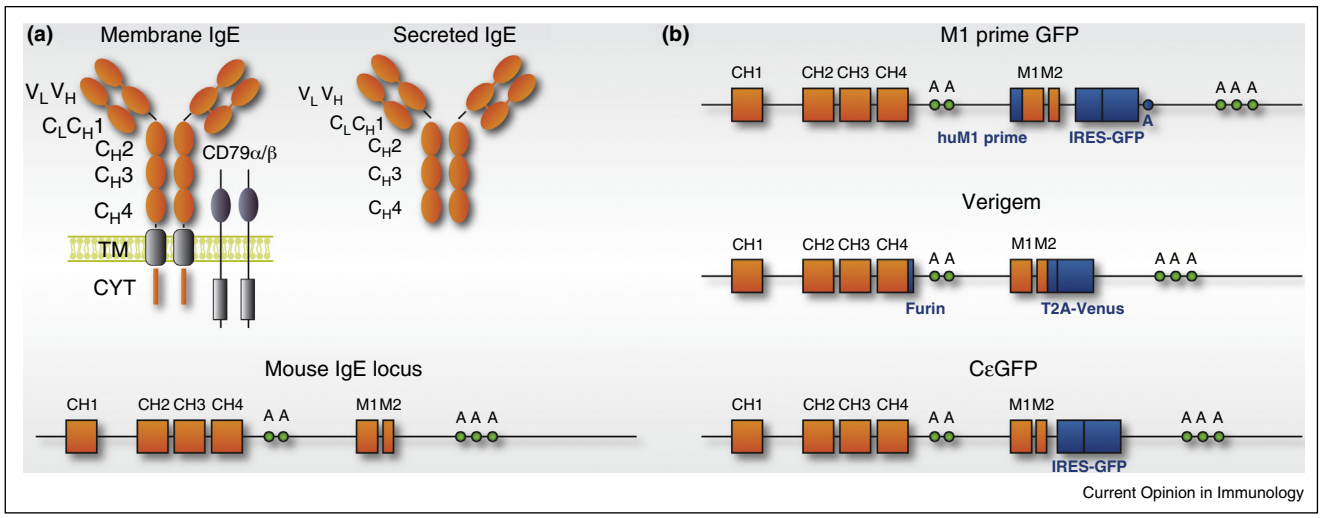
IgE production and memory in mice

IgE exists in two forms, a membrane BCR form that is expressed on IgE-switched B cells and a secreted form that is produced by IgE plasma cells (Figure 1a). Class switch recombination of naïve B cells to IgE-switched cells requires the cytokines IL-4 in mice and either IL-4 or IL-13 in humans [4,5]. Both primary and memory IgE antibody responses are generated through a pathway that requires membrane IgE-expressing cells, since these responses are absent in mice in which membrane IgE expression is abolished without affecting secreted IgE [6]. Consistent with this, mice in which the transmembrane and/or cytoplasmic domains of membrane IgE are modified have altered primary and memory IgE responses [6,7].

The pathway of B cell differentiation to IgE production, including the location and lifespan of IgE-producing plasma cells and the identity of the memory B cells that give rise to IgE memory responses, has been poorly understood due to difficulties in identifying IgE-switched B cells *in vivo* [8,9,10•,11•]. Recently, three separate groups have generated IgE reporter mice in which a fluorescent protein is associated with either transcription (M1 prime GFP knockin mice [12,13,14••,15,16] and CεGFP mice [17••]) or translation (Verigem mice [18••]) of the membrane IgE BCR (Figure 1b). Studies utilizing these reporter mice, as well as earlier studies that utilized mice with monoclonal T and B cells [19], have greatly increased the understanding of IgE production and memory and have revealed several mechanisms that limit IgE responses *in vivo* [10•,11•].

IgE antibody responses in mice are typically transient and are not sustained like IgG1 antibody responses [20,21]. Studies of Verigem mice revealed that early IgE responses are generated from short-lived IgE plasma cells located in extrafollicular foci. Late IgE responses arise from germinal centers, but in contrast to IgG1 germinal center B cells, which are sustained over time and which give rise to long-lived IgG1 plasma cells, IgE germinal center B cells do not persist and are predisposed to

Figure 1



The mouse IgE genomic locus and IgE reporter mice. **(a)** IgE exists in a membrane B cell receptor form, which associates with the alpha and beta subunits of CD79, and a secreted form. Both forms contain four immunoglobulin constant domains (CH1 to CH4), but the membrane form contains additional transmembrane (TM) and cytoplasmic (CYT) domains. The mouse IgE genomic locus consists of four exons encoding each of the heavy chain constant domains (CH1 to CH4), an exon encoding the transmembrane domain of membrane IgE (M1), and an exon encoding the cytoplasmic domain of membrane IgE (M2). There are two polyadenylation sites (A) associated with secreted IgE that are located between the CH4 and M1 exons, and three polyadenylation sites associated with membrane IgE that are located downstream of the M2 exon. The three polyadenylation sites downstream of the M2 exon contain suboptimal sequences that result in lower levels of membrane IgE transcript. **(b)** M1 prime GFP knockin mice contain an insertion of an IRES-GFP bicistronic reporter gene with an exogenous polyadenylation sequence downstream of the M2 exon of the membrane IgE gene, as well as an insertion of the M1 prime domain of human membrane IgE (a 52 amino acid N-terminal extension of the transmembrane M1 domain of human membrane IgE) into its comparable location in the mouse IgE locus [12]. Concerns have been raised that the insertion of the human M1 prime and/or the exogenous polyadenylation sequences may have affected membrane IgE expression or function in M1 prime GFP mice [13]. Since there were no differences in primary or memory IgE and IgG1 responses between wildtype and M1 prime GFP mice upon infection with *N. brasiliensis* or immunization with TNP-OVA, and there were also no differences in membrane IgE expression on *in vitro*-derived IgE-positive B cells in IgE switch cultures, it was concluded that these modifications did not significantly affect IgE or IgG1 responses or membrane IgE expression and function [12,15]. Verigem mice contain an in-frame insertion of a viral 2A peptide sequence and Venus yellow fluorescent protein at the end of the M2 exon of the membrane IgE gene [18••]. During protein translation, the 2A peptide causes a skip that ultimately yields two separate proteins: membrane IgE with an additional C-terminal 17 amino acid 2A peptide extension and the Venus protein. In addition, Verigem mice contain an insertion of a furin cleavage site at the 3′ end of the CH4 exon, which is a remnant of the original targeting vector. The modifications at the IgE locus in Verigem mice do not appear to significantly affect IgE responses or membrane IgE expression and function, since no significant differences in phenotype, differentiation, or behavior of IgE germinal center B cells or plasma cells were observed in Verigem mice compared to wildtype mice. CεGFP mice contain an insertion of an IRES-GFP bicistronic reporter gene downstream of the end of the M2 exon of the membrane IgE gene [17••].

differentiate into short-lived IgE plasma cells [18••]. Studies of M1 prime GFP knockin mice [14••,15] and CεGFP mice [17••] also demonstrated a transient IgE germinal center response and the generation of primarily short-lived IgE plasma cells, although the studies of CεGFP mice suggested that IgE germinal center B cells are predisposed to undergo apoptosis as opposed to differentiate into plasma cells. Thus, the persistence of IgE production in mice is limited by a transient germinal center response and a short lifespan of IgE-producing plasma cells.

Although most IgE plasma cells produced in mice are short-lived cells that reside in the lymph nodes and spleen, a small number of IgE plasma cells were found in the bone marrow in Verigem mice, M1 prime GFP knockin mice, and CεGFP mice [14••,17••,18••]. These cells are likely to be long-lived IgE plasma cells that contribute to low levels of sustained IgE antibody production, consistent with other studies that have identified long-lived IgE plasma cells in the bone marrow of wildtype mice [22,23].

Very little is known about the memory B cells that give rise to IgE memory responses. Studies of M1 prime GFP knockin mice have identified a small population of IgE-positive memory B cells and a small subpopulation of IgG1-positive memory B cells that give rise to IgE responses when transferred to B cell-deficient recipient mice and rechallenged with antigen [14••,16]. In these studies, it was calculated that the IgE memory B cells contributed to the majority of the IgE memory response. By contrast, studies of mice with monoclonal T cells and B cells [17••,19] have identified IgG1 memory B cells as the major source of IgE memory responses. In these studies, however, IgE and IgG1 memory B cells were

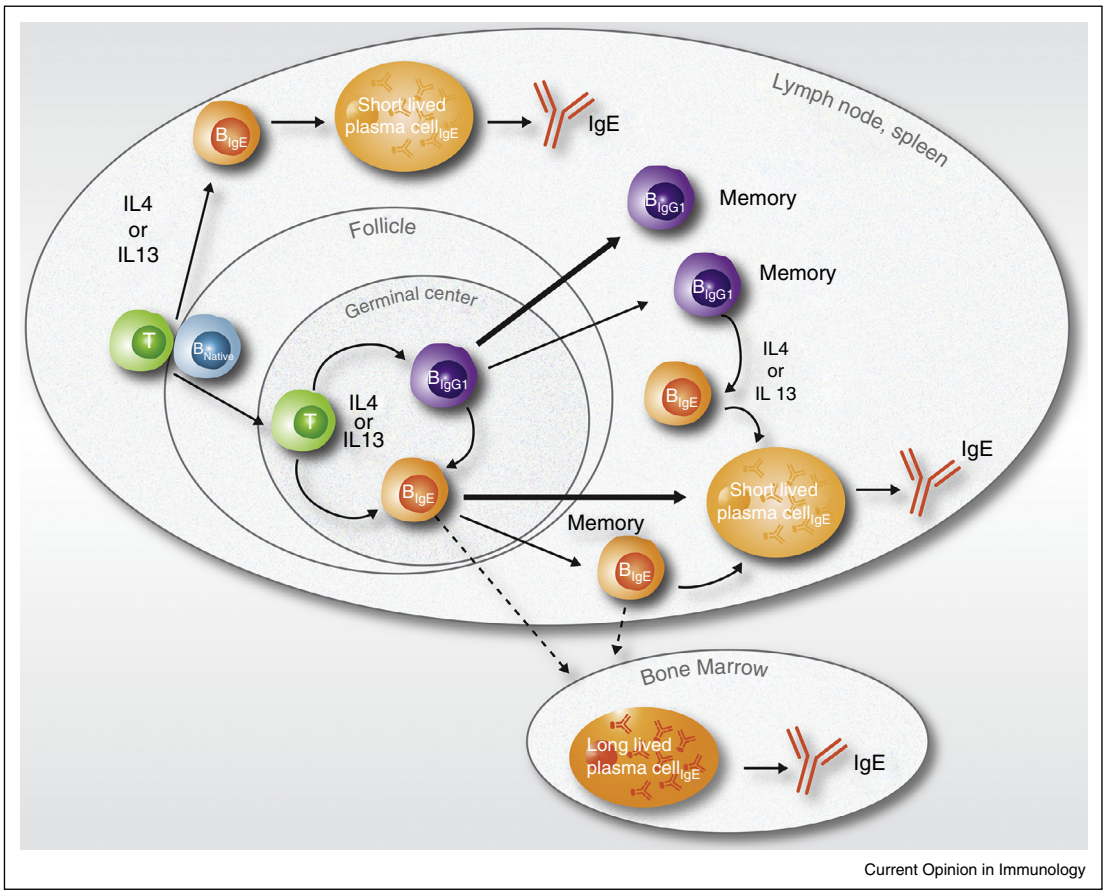
not purified and compared directly, and therefore it is possible that the contributions of IgE memory B cells were not fully accounted for due to their low frequency in the mixed cell populations that were examined. Overall, the understanding of the sources of IgE memory is limited and remains controversial.

Targeting IgE production

Taken together, the studies in mice have delineated a pathway of IgE production and memory that results in primarily transient, short-lived IgE antibody responses and limited IgE memory (Figure 2). This model for IgE production and memory suggests that a significant proportion of IgE antibody is generated from ongoing naïve and/or memory B cell activation and differentiation into IgE-producing plasma cells and implies that IgE antibody levels could be significantly reduced by inhibiting new IgE production, such as by targeting the cytokines IL-4 and IL-13 to inhibit IgE class switch recombination or by targeting IgE-switched B cells directly. In addition, this model also implies that a significant proportion of long-term IgE memory could be eliminated by targeting IgE-switched memory B cells, although the IgG1 memory B cells that contribute to IgE memory would not be affected by this approach.

Studies in mice and monkeys have shown that deficiency or neutralization of IL-4, IL-13, or the receptor IL-4Rα that is shared by both IL-4 and IL-13, inhibits IgE production [24–27], but only a few studies have assessed the effect of neutralization of IL-4/IL-13 during an ongoing or established IgE response [28]. A study in a cynomolgus monkey model of IgE responses to *Ascaris suum* antigen showed that treatment with anti-IL-13 antibodies over an 8-week period that included an *Ascaris* challenge resulted in a reduction

Figure 2



Model of IgE production and memory. IgE antibody is generated initially from extrafollicular B cell activation and differentiation into short-lived IgE plasma cells, and subsequently from germinal center B cell activation and differentiation. Class switch recombination of naïve B cells to IgE depends on the cytokines IL-4 and IL-13. Germinal center IgE responses are transient and generate primarily short-lived IgE plasma cells that reside in the lymph nodes and spleen, resulting in transient IgE antibody responses. A small number of long-lived IgE plasma cells are generated from germinal center reactions and can be found in the bone marrow, where they contribute to low levels of sustained IgE antibody production. IgE memory responses can arise from IgE memory B cells and a small subpopulation of IgG1 memory B cells that undergo a secondary switch to IgE upon reactivation, although the relative contributions of each of these memory B cell populations (as well as potentially other memory B cell populations such as IgM memory B cells) to total IgE memory responses remains controversial.

in *Ascaris*-specific IgE titers below pre-treatment levels, although no significant changes in total IgE levels were observed [25].

Multiple groups have directly targeted IgE-switched B cells using antibodies that bind either specifically to the membrane IgE BCR or to both membrane and secreted IgE [12,29–37], with several groups demonstrating *in vivo* activity of these antibodies [12,33–37]. Early studies showed that polyclonal and monoclonal anti-mouse IgE antibodies could inhibit primary and memory IgE responses, but did not prevent the development of IgE memory [35,36]. More recently, an antibody specific for mouse membrane IgE, which could trigger apoptosis of IgE B cells *in vitro*, inhibited IgE production when administered to mice preventively, but not when administered during an ongoing IgE response [34]. However, memory IgE responses in mice treated with this antibody during initial immunizations were significantly reduced long after treatment was stopped, suggesting that the antibody may have prevented the development of IgE memory B cells [34].

Antibodies targeting the M1 prime domain of human membrane IgE, which could trigger apoptosis and mediate antibody-dependent cell-mediated cytotoxicity of IgE B cells *in vitro*, inhibited both primary and memory IgE responses in M1 prime GFP knockin mice [12]. When administered during an ongoing IgE response in a mouse model of allergic asthma, these antibodies reduced antigen-specific IgE levels to levels comparable to those in naïve mice and far below the levels present at the initiation of treatment [12]. These antibodies also inhibited human IgE production in immunodeficient mice that were reconstituted with human immune cells [12,29]. In a different study, anti-IgE antibodies that bound both serum and membrane IgE were engineered for increased binding to the inhibitory IgG receptor FcγRIIb [33]. By binding both membrane IgE and FcγRIIb simultaneously on IgE-switched B cells, these antibodies inhibit membrane IgE signaling. When administered either preventively or during an ongoing IgE response in mice expressing a human FcγRIIb receptor or in immunodeficient mice reconstituted with human immune cells, these antibodies reduced IgE levels by greater than 90%. This *in vivo* activity required the co-engagement of membrane IgE with FcγRIIb.

Interestingly, two groups have reported high expression of membrane IgE on IgE plasma cells in mice [17••,18••], and therefore therapies that target membrane IgE-expressing cells may directly target not only IgE-switched B cells, but also IgE plasma cells. However, none of the studies discussed above determined the direct effect of the membrane IgE-targeted therapeutics on IgE plasma cells.

Effects of antibodies targeting IL-13, IL-4Rα, and the M1 prime domain of human membrane IgE on IgE production in clinical studies

It has been difficult to study IgE production in humans due to the low abundance of IgE-switched cells and technical limitations in identifying them. The limited available data on human IgE responses is largely consistent with what has been observed in mice. For instance, significant seasonal increases and decreases in allergen-specific and total IgE levels in allergic individuals, consisting of as much as two-fold changes observed over the course of several months, is reminiscent of the transient IgE responses observed in mice [38–40]. However, reports of long-term helminth-specific IgE [41] or the transfer of allergen-specific IgE to non-atopic recipients of bone marrow transplants [42,43] indicate that, in contrast to mice, there may be a significant contribution of long-lived IgE plasma cells to IgE production in humans. In addition, studies of patients with asthma and allergic rhinitis have described significant local IgE production in nasal and bronchial mucosal tissues [44], which has not been reported in mice.

Recently, therapeutic agents that target IgE production have been assessed in small Phase I and Phase II clinical studies (Table 1). These agents include therapeutics that target IL-4 (altrakincept), IL-13 (lebrikizumab, GSK67586, IMA-638, IMA-026, tralokinumab), IL-4Rα (dupilumab, AMG-317, pitrakinra), and membrane IgE (quilizumab). In reviewing the clinical data, it should be noted that differences in the effects of these therapeutic agents on IgE production may result from differences in the potencies of the various therapeutics against their respective targets, differences in therapeutic exposure due to different routes of administration and/or dosing frequencies, as well as differences in the characteristics of the patient cohorts in each clinical study.

The effect of neutralizing IL-13 and/or IL-4 on IgE production in humans has been assessed in a number of different clinical studies. Treatment with lebrikizumab, an anti-IL-13 monoclonal antibody, reduced total serum IgE levels by approximately 20% in patients with asthma [45•,46,47]. In these studies, proximal biomarkers of IL-13 blockade (e.g. FeNO and CCL17) revealed near-maximal inhibition of IL-13 activity following a single dose, whereas serum IgE levels declined more slowly during the 3–6 month treatment period. Since the half-life of serum IgE in humans is very short (approximately 1–2 days), these results are consistent with a slow decline in serum IgE upon the turnover of short-lived IgE plasma cells downstream of the inhibition of IL-13-induced IgE class switching. These studies also suggest that at least 20% of total serum IgE in these patients was generated from ongoing IgE B cell responses (which can be driven by both IL-4 and IL-13). By contrast, the anti-IL-13

Table 1

Summary of clinical studies.

Target	Therapeutic	Molecule	Asthma patient population	Treatment duration	IgE reduction relative to pre-treatment (mean %)	Reference
IL-4	Altrakincept (nebulized)	Soluble IL-4Rα protein	Moderate	12 weeks	No effect	[50]
IL-13	Lebrikizumab	Anti-IL-13 antibody	Mild; no ICS	13 weeks	24%	[47]
	Lebrikizumab	Anti-IL-13 antibody	Mild-moderate; no ICS	12 weeks	12%	[46]
	Lebrikizumab	Anti-IL-13 antibody	Moderate-severe, uncontrolled	24 weeks	22%	[45•]
	GSK67586	Anti-IL-13 antibody	Severe, uncontrolled	12 weeks	No effect	[48]
	IMA-638, IMA-026	Anti-IL-13 antibodies	Mild; no ICS	1 month	No effect	[49]
	Tralokinumab	Anti-IL-13 antibody	Moderate-severe, uncontrolled	13 weeks	No data available	[55]
IL-13 and IL-4	AMG-317	Anti-IL-4Rα antibody	Moderate-severe	12 weeks	49% (mean) 7% (median)	[52•]
	Dupilumab	Anti-IL-4Rα antibody	Moderate-severe, uncontrolled; ICS taper	12 weeks	37%	[53•]
	Pitrakinra (nebulized)	IL-4 variant protein	Atopic asthma	1 month	No effect	[51]
Membrane IgE (M1 prime domain)	Quilizumab	Anti-M1 prime antibody	Mild; no ICS	12 weeks	19%	[54••]

monoclonal antibodies IMA-638, IMA-026, and GSK67586 failed to demonstrate effects on serum IgE in clinical studies [48,49], but differences in antibody potencies, antibody exposure, and/or clinical study design may have contributed to the lack of effect as compared to lebrikizumab.

The contribution of IL-4 to IgE production in patients with asthma is less clear. Blockade of IL-4 using a soluble recombinant IL-4Rα protein (altrakincept) did not result in reductions in serum IgE, although this therapeutic was delivered via nebulization and therefore would have had only local effects in the lung, with very little systemic activity [50]. Similarly, blockade of both IL-4 and IL-13 using a nebulized variant IL-4 protein that binds to IL-4Rα but does not activate signaling (pitrakinra) did not have any effect on serum IgE [51]. By contrast, blockade of both IL-4 and IL-13 using monoclonal antibodies against IL-4Rα (AMG-317 and dupilumab) administered subcutaneously reduced total serum IgE levels [52•,53•]. Due to differences in patient populations and study designs, it is not possible to accurately assess whether blockade of IL-4 activity in addition to IL-13 activity by these anti-IL-4Rα agents had an incremental effect on reducing serum IgE compared to IL-13 blockade alone.

Quilizumab is an afucosylated monoclonal antibody against the M1 prime domain of human membrane IgE [29], which enables the direct therapeutic targeting of IgE-switched cells. The effect of quilizumab on IgE production has been assessed in three independent small phase I and II studies [54••]. In patients with mild asthma, quilizumab treatment completely inhibited new allergen-specific IgE production induced by whole lung allergen challenge [54••]. In addition, quilizumab treatment resulted in a gradual reduction in total serum IgE levels in healthy volunteers, patients with allergic rhinitis, and patients with mild asthma [54••]. The kinetics and extent of serum IgE reduction were similar following one or several dose administrations of quilizumab and were also similar to the reductions in total serum IgE observed upon blockade of IL-13 or IL-4Rα, consistent with this proportion of total serum IgE arising from short-lived plasma cells generated from ongoing IgE B cell responses. The residual total serum IgE levels that were not affected by quilizumab treatment may have been produced by long-lived IgE plasma cells that were not targeted by quilizumab. Interestingly, the reductions in total serum IgE were sustained at least six months after the last dose of quilizumab, suggesting that treatment with quilizumab may have abrogated some memory IgE responses that were contributing to ongoing IgE production, which were not regenerated upon the cessation of quilizumab therapy.

Conclusions

Studies of IgE production using genetically modified IgE reporter mice have revealed that most IgE in mice is produced by short-lived IgE plasma cells arising from ongoing IgE B cell responses. IgE responses in mice are transient, due to a limited persistence of IgE germinal center responses and the short life span of most IgE-producing plasma cells. IgE memory responses remain poorly understood, and the sources of IgE memory are controversial, although both IgE and IgG1 memory B cells have been implicated. Further studies of IgE production in mice are needed to better define the mechanisms that limit

IgE germinal center responses and predispose IgE-switched cells to differentiate into short-lived plasma cells, as well as the sources of IgE memory.

Results of clinical studies of agents targeting IL-4 and/or IL-13, as well as membrane IgE, indicate that a significant proportion of IgE in humans arises from short-lived IgE plasma cells and ongoing IgE B cell responses, similar to that observed in mice. However, the human clinical studies also suggest that a major proportion of IgE in humans, larger than that observed in mice, may arise from long-lived IgE plasma cells. It should be noted that differences in mouse models of IgE production compared to IgE production in humans may account for the differences in the effects of therapeutics in mice versus humans. For instance, mouse models of IgE responses are acute models with defined time points of immunization and therapeutic intervention. By contrast, patients treated with therapeutics have longstanding IgE responses arising from multiple previous allergic challenges, which may result in different proportions of short-lived versus long-lived plasma cells and differences in the extent to which therapeutic agents can reduce existing IgE levels. In addition, most mouse models employ one specific antigen/allergen for immunization and exposure, whereas allergic individuals typically have many different IgE specificities, some or all of which may contribute to disease pathogenesis.

Although the results of the clinical studies indicate that IL-13 plays an important role in IgE class switch recombination to generate IgE in humans, the contribution of IL-4 to IgE production remains to be clarified. Further studies are also needed to better understand the frequency and drivers of IgE class switching in humans, as well as the contribution of short-lived versus long-lived plasma cells to the total serum IgE pool in allergic patients, especially those with very high levels of IgE. In addition, studies are needed to define whether there are differential contributions of IgE generated from short-lived versus long-lived plasma cells, or from IgE produced in different anatomical locations, to disease pathogenesis in humans. An increased understanding of IgE production in health and disease may lead to new therapies for the treatment of allergic diseases.

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