# The who, where, and when of IgE in allergic airway disease

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**Allergic asthma and allergic rhinitis/conjunctivitis are characterized by a $T_H2$-dominated immune response associated with increased serum IgE levels in response to inhaled allergens. Because IgE is a key player in the induction and maintenance of allergic inflammation, it represents a prime target for therapeutic intervention. However, our understanding of IgE biology remains fragmentary. This article puts together our current knowledge on IgE in allergic airway diseases with a special focus on the identity of IgE-secreting cells ("who"), their location ("where"), and the circumstances in which they are induced ("when"). We further consider the therapeutic implications of the insights gained. (J Allergy Clin Immunol 2012;129:635-45.)**

***Key words:*** *IgE, atopic asthma, allergic rhinitis, memory B cells, plasma cells*

*Abbreviations used*

| | |
|---|---|
| Bcl-6: | B-cell lymphoma 6 |
| BCR: | B-cell receptor |
| Blimp-1: | B-lymphocyte maturation protein 1 |
| CD40L: | CD40 ligand |
| Cε: | Constant domain of IgE |
| CSR: | Class-switch recombination |
| DC: | Dendritic cell |
| GC: | Germinal center |
| GLT: | Germline transcript |
| mIg: | Transmembrane immunoglobulin |
| NGF: | Nerve growth factor |
| PC: | Plasma cell |
| SHM: | Somatic hypermutation |
| STAT: | Signal transducer and activator of transcription |
| $T_{FH}$: | Follicular helper T |

IgE, the fifth and least abundant class of immunoglobulins, is thought to have evolved in mammals from a first line of defense mechanism against parasites, particularly helminths and protozoa. However, today it is best known as a mediator of allergic reactions ranging from allergic rhinitis and asthma (Box 1) to life-threatening anaphylactic shock. IgE is composed of a pair of light chains and a pair of heavy chains (Fig 1). Like IgM, IgE has 4 heavy chain domains (Cε1-Cε4) and lacks a hinge region. IgE differs from other antibody isotypes in being located predominantly in tissues, where it is tightly bound to mast cells and basophils through its high-affinity receptor. In this cell-bound state IgE can persist for extended periods of time.[1] Most IgE is retained in tissues, and free serum IgE levels are the lowest of all immunoglobulin classes (50-200 ng/mL in healthy subjects compared with 10 mg/mL for $IgG_1$ and 3 mg/mL for $IgA_1$, Table I). In addition, its serum half-life is short: 2 days compared with 21 days for $IgG_1$ and 6 days for $IgA_1$. IgE does not activate complement and exerts its functions mostly through its receptors (Fig 2 and Box 2). The high-affinity receptor FcεRI is expressed on basophils and mast cells as an $\alpha\beta\gamma_2$ tetramer and on Langerhans cells, myeloid dendritic cells (DCs), plasmacytoid DCs, monocytes and eosinophils as an $\alpha\gamma_2$ trimer.[2-5] The low-affinity receptor FcεRII (CD23) is a C-type lectin and can be induced on a broad range of immune cells, such as activated B cells, macrophages, eosinophils, natural killer T cells, T cells, and follicular dendritic cells, but also on structural cells, such as airway epithelial cells and smooth muscle cells.[6]

## STATE OF THE ART ON IgE$^+$ B-CELL DEVELOPMENT

Typically, mature naive B cells encounter antigen in peripheral lymphoid organs, where it is presented by DCs. T-dependent antibody responses are initiated when rare B and T cells specific for an incoming antigen cluster at the boundary between B-cell follicles and T-cell zones and engage in cognate interactions. Activated B cells then can adopt one of 2 fates: movement into extrafollicular areas followed by proliferation and terminal differentiation into short-lived plasma cells (PCs) or movement into B-cell follicles followed by proliferation and establishment of germinal centers (GCs).[7]

Cognate $T_H$ cells interact with B cells and provide them with the necessary helper signals. The ligation of CD40, which is constitutively expressed on B cells, by CD40 ligand (CD40L), which is upregulated on activated $CD4^+$ $T_H$ cells, is known to play an essential role in differentiation, class-switch recombination (CSR), and GC formation.[8,9] Specialized follicular helper T ($T_{FH}$) cells, characterized by CXCR5, B-cell lymphoma 6 (Bcl-6), programmed death-1, and inducible costimulator expression, provide specialized B-cell help with a plethora of cytokines, such as IL-2, IL-4, IL-21, and TGF-β1.[10,11] Of these cytokines, IL-21 plays a particularly crucial role in GC formation by providing growth and differentiation signals and sustaining the expression of Bcl-6, a transcription factor necessary for GC B-cell development.[12,13] Among the different subtypes of $T_{FH}$ cells,

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**Box 1.** IgE in patients with atopic asthma and atopic rhinitis

Allergic asthma and allergic rhinitis/conjunctivitis are characterized by a $T_H2$-dominated immune response associated with increased serum IgE levels in response to inhaled allergens. Symptoms of atopic asthma are intermittent attacks of breathlessness, wheezing, and cough after exposure to inhaled allergens. These are caused by chronic inflammation and remodeling of the conducting airways. Atopic rhinitis/conjunctivitis is an inflammation of the nasal passages, usually associated with swelling, watery nasal discharge, and itching of the nose and eyes. The association between allergen-specific serum IgE levels and asthma was established through epidemiologic studies. Burrows et al[85] found a close correlation between serum IgE levels, skin test reactivity, and asthma. Involved antigens are mostly indoor aeroallergens derived from, for example, house dust mite, animal dander, cockroach, and molds. In patients with allergic rhinitis, seasonal allergens from pollens and grasses play an equally important role as aeroallergens.

Asthmatic patients who do not respond to common allergens on a skin prick test are defined as nonatopic. About a third of adult asthmatic patients are nonatopic by this definition. Nonatopic asthmatic patients usually have more severe and difficult-to-control disease. They also have increased total IgE serum levels compared with those seen in healthy control subjects.

Experimental asthma can be induced in animals in the absence of B cells or IgE,[86,87] suggesting that other immunologic effector pathways can induce allergen-driven airway inflammation.

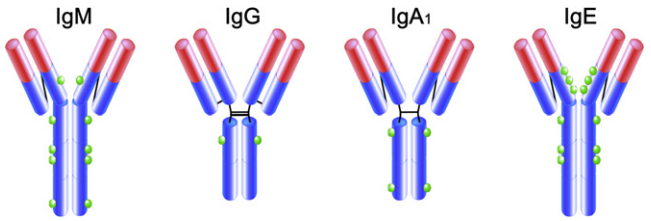


**FIG 1.** Protein structure of soluble IgE in comparison with IgM, IgG, and IgA. Schematic representation of the protein and domain structure showing the variable regions in *red* and the constant heavy chain regions in *blue*. Interdomain disulfide bridges are represented by *black lines*, and sites of N-linked glycosylation are represented by *green spheres*.

**TABLE I.** Human serum immunoglobulin levels and their main function

| Isotype | Serum concentration (mg/mL) | Serum half-life (d) | Main effector functions |
|---|---|---|---|
| IgD | 0.03 | 3 | Binding to mast cells and basophils<br>Neutralizing airway microbes |
| IgM | 1.5 | 10 | Classical pathway of complement activation<br>Neonatal immunity |
| IgG | | | |
| $IgG_1$ | 9.0 | 21 | Classical pathway of complement activation |
| $IgG_2$ | 3.0 | 20 | |
| $IgG_3$ | 1.0 | 7 | Fc receptor–dependent phagocytosis |
| $IgG_4$ | 0.5 | 21 | Neonatal immunity |
| IgA | | | |
| $IgA_1$ | 3.0 | 6 | Mucosal immunity: secreted into lumens of respiratory and gastrointestinal tracts<br>Alternative pathway of complement activation |
| $IgA_2$ | 0.5 | | |
| IgE | $5 \times 10^{-5}$ | 2 | High-affinity binding to mast cells and basophils (immediate hypersensitivity reactions) |

$T_H2$-like $T_{FH}$ cells are the only subset capable of inducing IgE class-switching in naive B cells.[14]

Once B cells have entered the GC reaction, they undergo clonal expansion and selection, antibody affinity maturation through somatic hypermutation (SHM), and CSR (Fig 3). Two signals are required for class-switching to IgE: IL-4/IL-13 and CD40 ligation.[15] This is illustrated by decreased IgE levels in mice deficient for CD40 or IL-4.[16,17] The $T_H2$ cytokines IL-4 and IL-13 are strong inducers of IgE switch through signal transducer and activator of transcription (STAT) 6, whereas CD40 ligation signals through nuclear factor κB. Both signaling molecules can bind the Iε promoter (STAT6 with a higher affinity) to initiate the production of "sterile" ε germline transcripts (εGLT).[18,19] Production of εGLTs from the Iε promoter precedes IgE class-switching and makes the ε switch region available for recombination. Nuclear factor κB and, to a lesser extent, STAT6,[20] induce expression of activation-induced cytidine deaminase, which initiates a series of reactions leading to recombination of the heavy chain between the Sμ and Sε regions.[21] This results in rearranged mature ε chain mRNA and a circular piece of DNA that is looped out, referred to as a switch circle. Both direct μ to ε CSR, as well as sequential switch through γ intermediates have been observed in human subjects,[22,23] as well as mice.[24]

An isotype-switched affinity-matured B cell exiting the GC reaction will become either a memory B cell or a long-lived PC. How this decision is made is not yet known. Memory B cells are long-lived, dividing B cells that carry their B-cell receptor (BCR) at high levels on the surface and secrete little immunoglobulin.[7] On antigen recall, they react rapidly and give rise to antigen-specific antibody-secreting PCs. PC precursors or plasmablasts that emerge from GC reactions are highly proliferative cells and migrate primarily to the bone marrow, where they terminally differentiate into PCs. They downregulate several B-cell lineage–specific markers and upregulate the PC marker CD138 (syndecan-1). The transcription factors B-lymphocyte maturation protein 1 (Blimp-1) and X-box protein 1 are necessary for PC development because they regulate secretory processes and endoplasmic reticulum stress, allowing the endoplasmic reticulum machinery to adapt to high-level antibody production. Long-lived PCs provide long-term antibody titers; they do not self-renew and express very low to undetectable levels of membrane immunoglobulin.[25] Together, memory B cells and long-lived PCs ensure humoral memory. In the case of IgE, it is not clear whether both long-lived PCs and memory B cells exist. Fig 4 provides an overview of immunoglobulin expression, membrane markers, and transcription factors in the different stadia of B-cell differentiation.

## OUTSTANDING QUESTIONS/GAPS IN OUR KNOWLEDGE OF IgE BIOLOGY

**"Who"**: Is IgE memory ensured by PCs and memory B cells?

**"Where"**: Where do IgE-secreting cells reside?

"**When**": Does IgE CSR take place during GC formation? Can it happen independently of T-cell help?

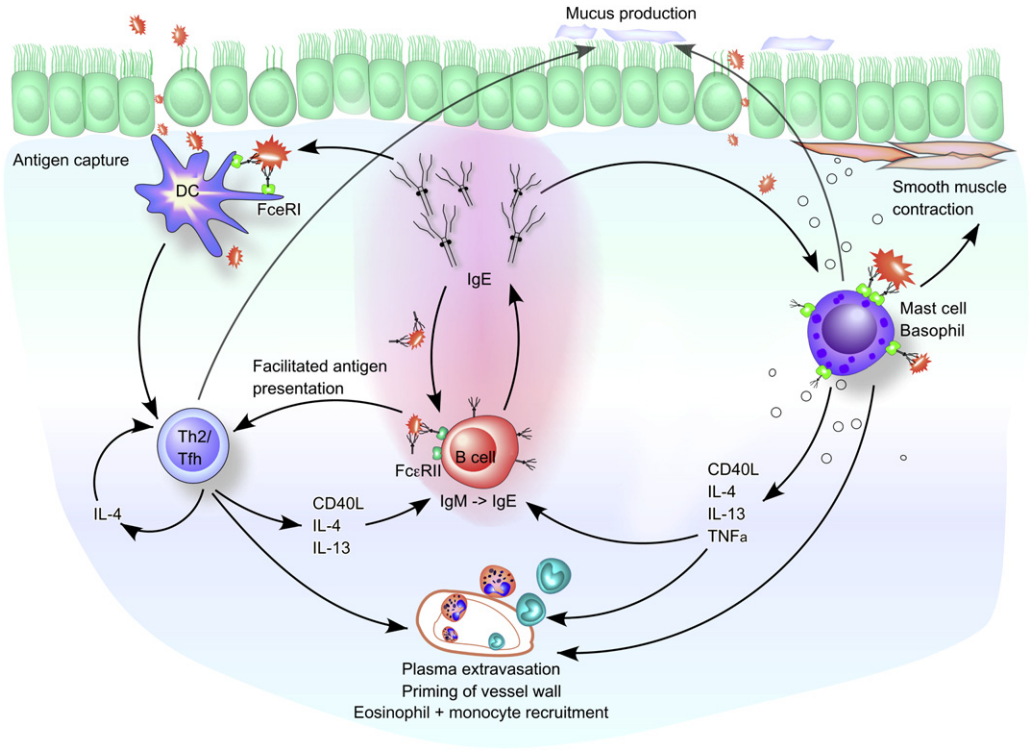


**FIG 2.** Central role of IgE in patients with allergic airway diseases. When DCs take up inhaled antigens in an atopic environment, this results in a predominant $T_H2$ cell induction. In addition to recruiting eosinophils, $T_H2$ cells are potent inducers of $IgE^+$ B cells through production of IL-4 and IL-13. IgE produced locally in the mucosa binds to its high-affinity (FcεRI) and low-affinity (FcεRII) receptors on cells of the airway mucosa. Ligation of FcεRI-bound IgE by allergen on mast cells and basophils leads to degranulation and the synthesis of inflammatory mediators and cytokines, which cause the symptoms of immediate hypersensitivity, such as plasma extravasation, smooth muscle contraction, and itching. Release of proinflammatory (IL-6, macrophage inflammatory protein 1α, and TNF-α) and type 2 (IL-4, IL-5, and IL-13) cytokines initiates the late-phase response with recruitment and activation of inflammatory cells, such as monocytes and eosinophils. IL-4 and IL-13 also cause goblet cell hyperplasia and excess mucus production. Increased local IgE levels induce the expression of FcεRI on DCs and stabilize FcεRII on B cells. This allows both cell types to take up allergen-IgE complexes and present the allergen to T cells in a $T_H2$-skewing environment. For FcεRII-bearing B cells, this happens in a noncognate manner, and it is therefore called facilitated antigen presentation.

**Box 2.** Effector functions of IgE (Fig 2)

IgE produced locally in the mucosa binds to its high- and low-affinity receptors on a wide range of immune cells and structural cells of the airway mucosa. Mast cells and basophils are the best-known IgE effector cells and are responsible for the acute phase of airway inflammation. Cross-linking of IgE-FcεRI complexes on the mast cell and basophil surface leads to degranulation and synthesis of inflammatory mediators and cytokines. Histamine, leukotrienes, prostaglandins, and platelet-activating factor cause the symptoms of immediate hypersensitivity, such as vascular permeability, blood vessel dilation, bronchoconstriction, plasma extravasation, and smooth muscle contraction. Release of proinflammatory (IL-6, macrophage inflammatory protein 1α, and TNF-α) and type 2 (IL-4, IL-5, and IL-13) cytokines initiates the late-phase response with recruitment and activation of inflammatory cells, such as monocytes, T cells, eosinophils, and basophils. These cells contribute to the clearance of allergen-IgE complexes and phagocytose and kill pathogens.[6] In addition to promoting more IgE production, IL-4 and IL-13 also cause goblet cell hyperplasia and excess mucus production.[88,89]

Expression of FcεRI on antigen-presenting cells is important for IgE-mediated antigen uptake. Allergen that binds DCs through FcεRI-fixed specific IgE is efficiently taken up and presented to T cells, thus amplifying the late allergic response.[90] In addition, $FcεRI^+$ DCs have been shown to be necessary and sufficient for the induction of $T_H2$-driven allergic airway inflammation in response to inhaled house dust mite.[5]

CD23 is upregulated on antigen-engaged B cells and allows them to bind allergen-IgE complexes, internalize the allergen, and present its peptides to T cells in a noncognate manner, a process also called facilitated antigen presentation.[91] CD23 expressed on epithelial cells acts as a transepithelial IgE-allergen transporter, transferring IgE-allergen complexes from the lumen to the mucosa, where they can engage FcεRI on mucosa-resident mast cells.[92] Ligation of CD23 by IgE-allergen complexes on airway smooth muscle cells induces production of IL-1β, which contributes to inflammation.[93]

IgE-mediated antigen presentation by both B cells and DCs amplifies the allergen-specific IgE response. Repeated allergen exposure thus creates an IgE-driven chronically inflammatory environment causing tissue damage that will engage wound repair mechanisms. These repair mechanisms will, in the case of asthma, eventually result in remodeling of the airways characterized by goblet cell hyperplasia, increased airway wall thickness from smooth muscle hyperplasia, collagen deposition, and increased vascular permeability.[94]

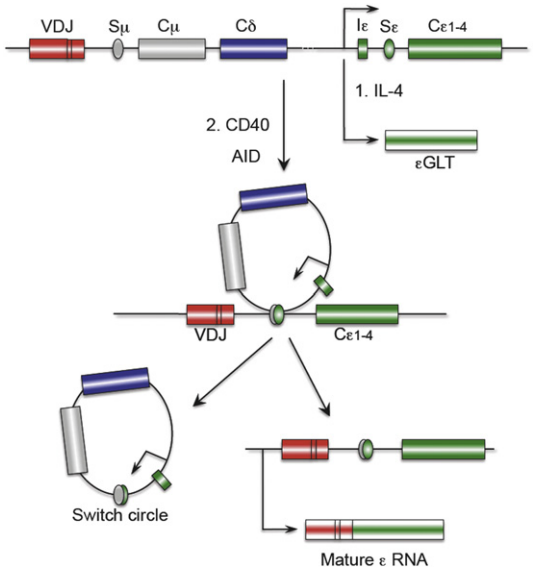


**FIG 3.** Molecular events leading up to IgE class-switching. Schematic representation of the immunoglobulin heavy chain locus with regions of interest. A mature B cell expresses a rearranged VDJ coupled to Cμ and Cε. IgE class-switching is initiated by IL-4 through induction of STAT6 and by CD40 ligation through translocation of nuclear factor κB. These signaling molecules initiate transcription from the Iε promoter (εGLT) and induce expression of activation-induced cytidine deaminase *(AID)*. This enzyme catabolizes a deletion recombination when the switch regions Sμ and Sε pair up their homologous sequences. The result is a genomic sequence in which the VDJ regions are followed by the Cε region, giving rise to mature ε transcripts.

## "WHO": IS IgE MEMORY ENSURED BY BOTH PCs AND MEMORY B CELLS?

The formation of allergen-specific IgE in atopic subjects often occurs early in life[26] and usually continues long into adulthood, even without continuous antigen exposure.[27] Studies on allergen-specific IgE reactivity in allergic subjects found that it is steady and exhibits characteristics of a typical secondary (ie, memory) response.[28,29] In a murine model IgE transcripts could be detected in the spleen only 1 day after boost, indicating that IgE memory B cells exist.[30] More in-depth research is hampered by the extreme low frequency at which these cells occur.

In human subjects switched transmembrane immunoglobulin (mIg) $G^+$ and $mIgA^+$ memory B cells can be found recirculating through the blood. On average, they represent 9% and 6%, respectively, of circulating B cells in healthy subjects (Fig 5, *A*), which is in line with serum IgG and IgA levels. Given that serum IgE concentrations are 4 to 5 orders of magnitude lower than those of IgG, it is expected that $mIgE^+$ B cells would be near undetectable in the blood.

In our hands, using flow cytometry, the blood of healthy and mildly atopic subjects contained comparable $mIgE^+$ B-cell numbers (0.1% to 0.2% of peripheral blood B cells; Fig 5, *A* and *B*). Allergic patients with increased serum IgE levels (>1000 kU/mL) had only slightly increased frequencies of $mIgE^+$ B cells (mean, 0.47%), which is in line with results by Donohoe et al (Table II). Serum IgE concentrations did not correlate well to $mIgE^+$ B-cell frequency but did correlate to IgE fluorescence intensity on circulating basophils, monocytes, and plasmacytoid dendritic cells, the effectors of allergic reactions (Fig 5, *C*).

The short half-life of serum IgE implies that a constant *de novo* production must occur to sustain allergen-specific IgE levels. It is known that long-lived PCs are responsible for the long-term maintenance of antigen-specific antibody titers.[31,32] This is most likely also the case for IgE because it is known that atopy can be transferred through transplantation of bone marrow from an atopic into a nonatopic patient.[33] Moreover, it has been shown in mice that the majority of IgE-secreting cells after airway allergen exposure reside in the bone marrow and are long-lived and cyclophosphamide resistant, which is typical of long-lived PCs.[34] Direct proof of the existence of circulating human IgE-secreting PCs was provided by a number of studies (Table II). To summarize, $IgE^+$ PCs were detected by using ELISPOT in the blood of patients with increased serum IgE levels but were on the lower end of detection in healthy subjects. The frequency of circulating IgE-secreting cells correlates well with serum IgE levels in all studies.

On the basis of their findings in a murine model, Erazo et al[24] claim that $IgE^+$ B cells exhibit a unique maturation program favoring swift PC differentiation over memory B-cell development. In their model $IgE^+$ B cells developed through sequential switch from $IgG_1{}^+$ B cells in the extra-GC areas. They found that $IgE^+$ B cells did not express the GC B-cell transcription factor Bcl-6 but instead contained high levels of the PC-related transcription factors Blimp-1 and X-box protein 1. Because these data could be a unique feature of the monoclonal T-cell/B-cell murine model used in this study, further research in more physiologic animal models and human allergic patients is warranted to validate the premise that $IgE^+$ B cells preferentially develop into PCs.

Taken together, our knowledge on the cellular source of IgE memory is fragmentary, but both PCs and memory B cells have been detected in the blood. With regard to IgE-targeting therapeutics under development, it is of major interest to further characterize each subset and determine their relative contribution to long-term IgE production. This is possible by studying the fate of $IgE^+$ B cells in more relevant animal models (ie, by using real allergens in wild-type mice and follow-up in atopic patients or patients with hyper-IgE syndrome).

## "WHERE": WHERE DO IgE-SECRETING CELLS RESIDE?

Where does the IgE come from that causes allergic reactions at mucosal sites? One hypothesis is that it is produced in regional lymph nodes or in the bone marrow and specifically enriched in the mucosa through binding to resident mast cells. Alternatively, IgE could be produced locally in the mucosa by resident PCs.

Luger et al[34] found in a murine model that long-lived allergen-specific PCs persisted in the bone marrow and spleen long after allergen exposure had stopped. Together with the fact that bone marrow transplantation in patients can transfer atopy,[33] this supports the theory that memory IgE PCs persist in the bone marrow.

Ganzer and Bachert[35] found IgE-containing lymphoid follicles in human palatine and nasopharyngeal tonsils and in cervical lymph nodes. They therefore suggested that IgE synthesis takes place in the lymphoid tissues of the Waldeyer ring and in lower respiratory tract lymph nodes, after which it is transported to the respiratory mucosa by migratory mast cells that mediate the allergic reaction. In line with this, McMenamin et al[36] identified in rats the lymph nodes draining the lower respiratory tract as the primary site of IgE secretion in response to inhaled antigen.

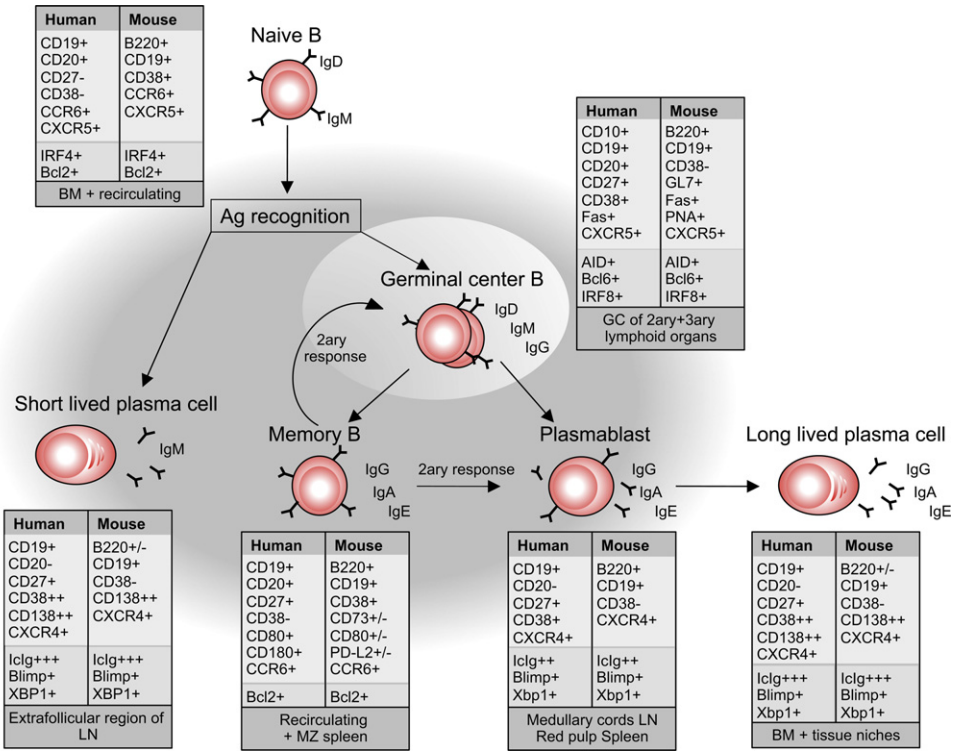


**FIG 4.** Markers of B-cell differentiation on antigen recognition. Schematic representation of B-cell differentiation on antigen recognition in human subjects and mice and the markers that characterize the different stadia are shown. Membrane markers are shown in the *upper box*, intracellular immunoglobulin and transcription factors are shown in the *middle box*, and the location of the subset is shown in the *lower box* per differentiation stage. *Ag*, Antigen; *BM*, bone marrow; *IcIg*, intracellular immunoglobulin; *LN*, Lymph node; *MZ*, marginal zone.

Yet in Northern blot analysis, they picked up ε chain mRNA in the lung parenchyma and in tracheal tissue, as well as in lung-draining lymph nodes, suggesting that IgE can be produced in the mucosa as well.

This had already been suggested, when allergen-specific IgE was found in nasal secretions of patients with allergic rhinitis, even if they had a negative skin prick test response.[37,38] Indeed, IgE-producing cells and IgE-encoding mRNA were found in nasal mucosa and nasal lavage fluid from both patients with atopic rhinitis and healthy subjects, but they were several times more abundant in the allergic subjects.[39-41] Allergen-specific $IgE^+$ PCs were found only in allergic patients and correlated to their atopic status.[41] It was further shown that *de novo* grass pollen–specific IgE synthesis takes place in the nasal mucosa of patients with hay fever and that this persists between seasons.[42] High levels of local IgE and $IgE^+$ B cells were also found in nasal polyps, independent of the atopic status of the patient.[43,44] In parallel, asthmatic patients were found to have increased levels of IgE-encoding mRNA in the bronchial mucosa compared with those seen in healthy control subjects, and this was irrespective of their atopic status.[23,45] Thus it appears that allergen-specific $IgE^+$ B cells and PCs accumulate in the respiratory mucosa of atopic subjects. Luger et al[34] found in a murine model that persistence of mucosal $IgE^+$ cells was dependent on continuous allergen exposure. This might be different in human subjects, however, because Smurthwaite et al[42] found pollen-specific IgE being produced in the allergic nasal mucosa between seasons.

The mucosa as the principal site of allergen-specific IgE production could explain the difference between so-called nonatopic and atopic patients: a negative skin prick test response or lack of allergen-specific IgE in the serum does not exclude the presence of allergen-specific IgE at the mucosal surface. Hence it is conceivable that many "nonatopic" patients are atopic after all but that they do not have spillover of the mucosally produced allergen-specific IgE into the circulation.

An important question with regard to therapeutic intervention that remains unanswered is whether $IgE^+$ B cells at the respiratory mucosal surface are continuously replenished from a source of local progenitors or whether the mucosa is merely a survival niche for cells induced elsewhere? *In vitro*–generated human $IgE^+$ B cells express CCR10 and migrate toward CCL28, which is produced by the airway epithelium on inflammation.[46] This would enable $IgE^+$ B cells that were generated elsewhere to migrate into inflamed airways. Murine studies further showed that adoptively transferred allergen-pulsed DCs migrated from the lung tissue to draining lymph nodes and subsequently induced allergen-specific IgE.[47] Together with the abovementioned studies,[35,36] this shows that respiratory tract draining lymph nodes contribute to IgE induction.

On the other hand, it has become increasingly clear that IgE class-switching can occur within lymphoid accumulations in the mucosa, as reviewed by Gould et al.[48] Several groups have found increased IL-4, signal 1 for IgE-class switching, in the nasal mucosa of patients with allergic rhinitis[49] and bronchial biopsy

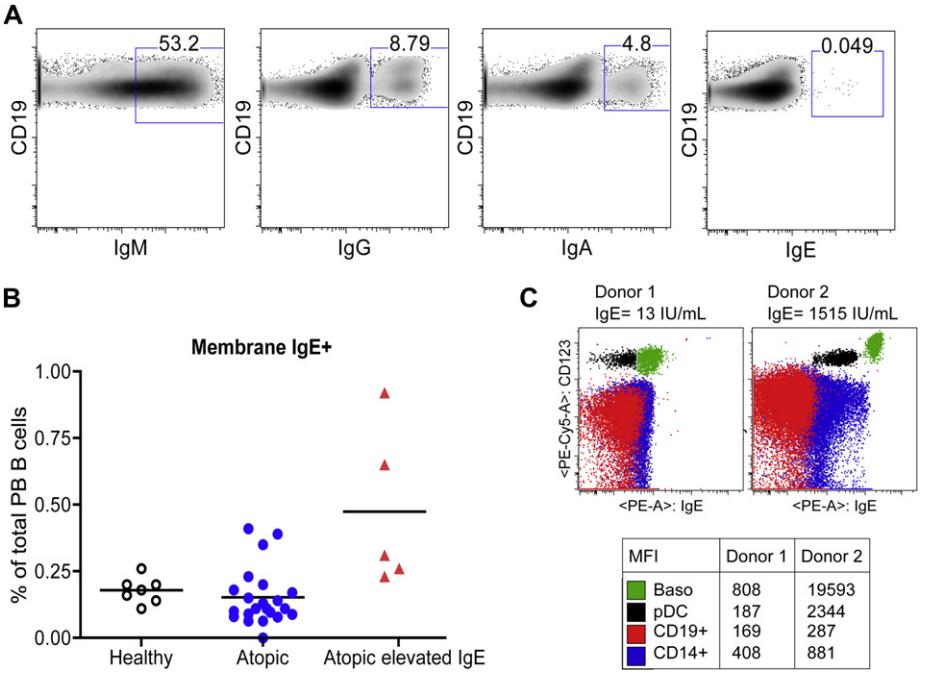


**FIG 5.** Circulating mIgE$^+$ B cells are not readily detected in the blood of allergic donors. Data are flow cytometric analyses of PBMCs from healthy subjects or patients with house dust mite allergy. Staining for CD19 and membrane immunoglobulins was performed. **A,** Data from a representative donor with house dust mite allergy for membrane IgM, IgG, IgA, and IgE levels. Cells were gated on scatter, singlets, alive, and CD19. Data from all donors tested are plotted in **B**. **C,** Although there are very few B cells expressing mIgE, basophils, plasmacytoid DCs, and monocytes bound IgE relative to the serum IgE levels. Populations were gated as follows: basophils, CD123$^+$BDCA2$^-$; plasmacytoid DCs, CD123$^+$BDCA2$^+$; monocytes, CD14$^+$.

**TABLE II.** Membrane IgE$^+$ and IgE-secreting cells in human blood

| Publication | Method | Subjects (serum IgE levels) | Frequency |
|---|---|---|---|
| Membrane IgE$^+$ cells in human blood | | | |
| Donohoe, 1995[98] | Flow cytometry | Healthy | 0.05% of PB B cells |
| | | Atopic | 0.33% |
| Dullaers et al, unpublished | Flow cytometry | Healthy (<150 IU/mL) | 0.18% of PB B cells |
| | | Atopic (150-500 IU/mL) | 0.15% |
| | | Atopic increased IgE level (>1,000 IU/mL) | 0.47% |
| | | | |
| IgE-secreting cells in human blood | | | |
| Stein, 1986[99]; MacKenzie, 1989[100] | EBV transformation + IgE ELISA on clone supernatant | Healthy | 0.03% to 0.1% of EBV-responsive B cells |
| Dhanjal, 1992[101] | ELISPOT | Healthy (<100 IU/mL) | ND |
| | | Atopic (<1,000 IU/mL) | ND |
| | | Atopic dermatitis (>2,000 IU/mL) | 49 IgE AFCs/$10^6$ PBMCs |
| King, 1991[102] | ELISPOT | Healthy (>200 ng/mL) | 0.4 IgE AFCs/$10^5$ PB B cells |
| | | Loiasis (200-13,500 ng/mL) | 5 |
| | | Lymphatic filariasis (350-26,000 ng/mL) | 9 |
| | | Tropical pulmonary eosinophilia (2,300-84,400 ng/mL) | 52 |
| | | Hyper-IgE syndrome (14,400-72,700 ng/mL) | 218 |
| Horst, 2002[103] | CD138 enrichment + intracellular flow | Healthy | 0.32% of PB PCs |
| | | Hyper-IgE syndrome | 6.5% |

*AFC*, Antibody-forming colony; *ND*, not detected; *PB*, peripheral blood.

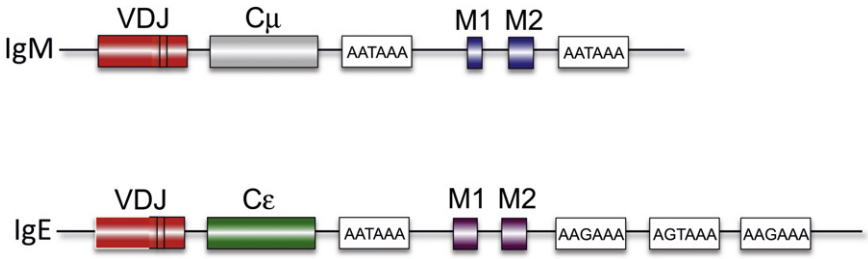


**FIG 6.** The IgE membrane regions possess 3 suboptimal termination sequences. Schematic representation of the IgM and IgE sequences showing that the membrane regions M1 and M2 of IgE are followed by 3 deviant termination sequences, unlike IgM. This results in a suboptimal expression of membrane IgE in favor of sIgE.

**Box 3.** Inefficient splicing favors the secreted form of IgE (Fig 6)

Like the other immunoglobulin classes, IgE is produced as a transmembrane (mIgE) or a secreted (sIgE) protein. Expression of the 2 forms is regulated by alternative splicing of the immunoglobulin heavy chain transcripts. The constant domains of the heavy chain are followed by 2 additional exons for the transmembrane domain (M1) and the cytoplasmic tail (M2, Fig 6). The last constant-domain exon of all immunoglobulin heavy chain genes contains an internal polyadenylation site and a splice donor site. The 3′ untranslated region downstream of M2 contains 1 or more external polyadenylation sites (Fig 4). Alternative processing of this transcript is regulated by the choice of polyadenylation sites and determines whether M1 and M2 are included in the mature mRNA. The membrane immunoglobulin/sIg ratio depends on the differentiation stage of the B cell, but the exact biochemical signals that regulate this process are not known.[95]

Both in mice and human subjects, the IgE membrane locus contains 3 poly-A termination sequences that deviate from the consensus AATAAA sequence found in other immunoglobulin genes (Fig 6).[96] As a result, the expression of mRNA for mIgE but not sIgE is poor because of inefficient processing at the deviant polyadenylation hexamers, and this leads to the preferential synthesis of sIgE. However, mice expressing a truncated ε cytoplasmic tail (hence signaling downstream of their BCR is defective) have significantly lower serum IgE levels after immunization and the antibodies are of a lower affinity compared with wild-type mice.[97] This means that mIgE, although expressed inefficiently, does play a role in positive selection and affinity maturation of $IgE^+$ B cells.

specimens from asthmatic patients.[50] It is known that mast cells present in airway mucosa can provide CD40L, signal 2 for IgE switching.[51] Furthermore, activation-induced cytidine deaminase expression and levels different IgE CSR-related nucleic acid species were increased in the nasal mucosa of patients with allergic rhinitis[23,52] and in the bronchial mucosa of atopic asthmatic patients compared with those seen in healthy control subjects.[53] Together these data demonstrate that the respiratory mucosa has the intrinsic capacity to drive local IgE class-switching.

Additionally, murine models of asthma have shown that the respiratory mucosa provides survival signals for resident PCs. Airway epithelial cells are a major source of the neurotrophins nerve growth factor (NGF) and neurotrophin-2 in patients with allergic airway inflammation.[54] Lung PCs express receptors for NGF and neurotrophin-3 (TrkA and TrkC).[55] Blocking of the NGF pathway resulted in lower allergen-specific IgE levels because of increased cell death of PCs,[55] whereas overexpression of NGF in Clara cells increased allergen-specific IgE levels and airway eosinophil numbers.[56]

In summary, IgE-secreting PCs are found in the bone marrow, respiratory lymph nodes, and respiratory mucosa. Both draining lymph nodes and lymphoid accumulations within the mucosa are IgE-inducing sites. The lung provides a survival niche for PCs by means of local production of neurotrophins, such as NGF.

## "WHEN": IS GC FORMATION NEEDED FOR IgE SWITCHING?

Even though it is clear that B cells need T cell–provided CD40L and IL-4 for switching to IgE, there is disagreement over the importance of the GC reaction for the generation of $IgE^+$ B cells. The observation that increased levels of IgE in germ-free rats normalized on nonsterilized chow feeding, which was associated with the emergence of GCs in Peyer patches, led to a hypothesis that the GC reaction itself is a negative regulator of IgE CSR.[57]

Looking at the location of $IgE^+$ B cells, some animal studies found that postimmunization $IgE^+$ B cells were localized exclusively in the GCs of draining lymphoid tissues,[58,59] whereas others observed that they were excluded from the GCs.[24,60] The most recent of these studies used an elegant murine model to visualize IgE responses.[24] Even though they found $IgE^+$ B cells primarily outside of GCs as opposed to $IgG_1{}^+$ B cells, they detected increased levels of εGLTs in GC B cells, suggesting that IgE CSR was initiated in GCs.

Aalberse and Platts-Mills[61] hypothesized that IgE is primarily induced when allergen exposure is low, such as with pollen or house dust mite, which results in a $T_H2$ response that is too weak to induce mature GCs or $T_{FH}$ cells.[10] In this case the absence of the GC B-cell transcription factor Bcl-6 would lead to rapid induction of Blimp-1 and plasmablast differentiation without formation of memory B cells. Because these responses are inefficient, the resulting antibody response is low, does not provide long-lived memory, and depends on chronic allergen stimulation to last.

To the contrary, high-dose allergen exposure, as with cat allergen or bee venom, induces strong $T_H2$ responses and mature GCs. Here, $IgE^+$ B cells have a survival disadvantage compared with other isotypes. Not only does inefficient expression of membrane IgE (Fig 6 and Box 3) make it more difficult for $IgE^+$ B cells to receive survival signals through their BCRs, interaction of membrane IgE with follicular DC-expressed CD23 can also suppress their survival.[62] This results in a $T_H2$-driven GC response,

generating predominantly $IgG_4$ B cells (in human subjects) that differentiate toward both PCs and memory B cells, as has been found in children exposed to cat allergen or beekeepers exposed to multiple bee stings.[63] In these strong $T_H2$ GC reactions, $IgE^+$ PCs could develop either through direct μ to ε CSR and escape from the follicle during early GC reaction or through sequential CSR from $IgG_4$ B cells. This hypothesis fits very well with Erazo et al's experimental data[24] and could also explain how the different models discussed above could have obtained such different results.

Dahlke et al[64] proposed that $IgE^+$ B cells would develop from a pathway distinct from the conventional GC pathway because they found that IgE $V_H$ sequences isolated from the blood of healthy and allergic subjects accumulated few somatic point mutations compared with IgG sequences. However, studies looking at SHM in mucosal IgE sequences of allergic subjects readily showed that antigen-driven SHM is an ongoing process in the mucosa, as documented by a mutation rate of 5% to 15%.[65,66]

Taken together, evidence from different models and patients suggests that $IgE^+$ B cells are dependent on GCs but not in a conventional way like, for example, $IgG^+$ B cells.

## "WHEN": CAN IgE CSR HAPPEN INDEPENDENTLY OF T-CELL HELP?

In addition to unconventional GC-dependent pathways, alternative T-independent mechanisms have been suggested to give rise to IgE production.

A preferential use of the minor $V_H5$ family by IgE was observed in the peripheral blood and spleens of atopic asthmatic patients, as well as in the nasal mucosae of patients with allergic rhinitis.[67,68] This biased $V_H$ gene use points toward selection by bacterial superantigens, which can bind immunoglobulins through the conserved framework region outside the conventional binding sites in the complementarity determining regions. Indeed, Coker et al[69] found a preferential $V_H5$ use for IgE and IgA sequences in the nasal mucosa and that 75% of mutational hot spots were located in the framework region. Because it is known that nasal carriage of *Staphylococcus aureus* occurs in more than a third of the population[70] and is associated with house dust mite–induced allergic rhinitis[71] and nasal polyposis,[72] bacterial superantigens are likely to play a role in airway mucosa IgE responses. It remains to be determined how superantigens at the mucosal surface favor IgE responses over other isotypes.

Another nonclassical pathway of IgE production was observed in CD4-deficient mice. As in human T-cell deficiencies accompanied by increased levels of serum IgE (eg, Wiskott-Aldrich and Omenn syndromes), McCoy et al[73] observed increased levels of spontaneously sIgE in the sera of mice from CD4-deficient strains and limited CD4-repertoire mice. This "natural" IgE arises in the absence of cognate CD4 T-cell help and, like natural IgM, is composed of natural specificities. It does not require secondary lymphoid structures or GC formation, although some bystander T cell–derived IL-4 is necessary. Recently, it was shown that $lck^{-/-}$ mice, which lack $T_H2$ cells, have paradoxically high IgE levels and that this is caused by an increase in $CD4^+$ γδ T-cell numbers.[74] It is thus highly likely that increased IgE levels in the other CD4-deficient mice are also a result of γδ T-cell expansion.

Another pathway to natural IgE could involve IgD cross-linking on basophils. It was recently shown that the human upper respiratory mucosa gives rise to IgD-switched plasmablasts that secrete IgD locally.[75] This soluble IgD recognizes several strains of respiratory bacteria and can be bound by basophils and mast cells. Cross-linking of IgD bound on the surfaces of basophils results in their activation accompanied by the secretion of antimicrobial factors and upregulation of, for example, CD40L, B cell–activating factor of the TNF family (BAFF), and IL-4. This enabled basophils to induce IgG and IgA CSR in naive B cells. However, it would be most interesting to know whether IgD-activated basophils and mast cells would trigger IgE CSR. This could provide a mechanism for the strong IgE responses induced in mice by a single injection of polyclonal anti-IgD antibodies, as reported by Finkelman et al.[76,77] It could also partially account for the IgE produced in the CD4-deficient mice because the majority of B cells in these mice would not be able to class-switch and hence produce IgD. Additionally, the presence of numerous IgD-secreting cells and mast cells might contribute to the intrinsic IgE-promoting properties of the respiratory mucosa.

In conclusion, in some circumstances IgE production can arise from alternative T-independent pathways. This is an emerging field of study, and more research is warranted to establish the importance of bacterial superantigens, natural IgE, and basophil IgD-cross-linking in the cause of allergic diseases.

## THERAPEUTIC IMPLICATIONS

In many ways the development of $IgE^+$ B cells differs from that of $IgG^+$ or $IgA^+$ B cells. In-depth study of the "who, where, and when" of IgE leads us to 3 main observations with important therapeutic implications.

### Local production, local treatment

IgE induction seems to occur mainly in the respiratory mucosa, and receptor-bound IgE is more abundant in the mucosa than in the circulation. This indicates local rather than systemic treatment. Not only would local treatment be more effective, it would allow for lower drug doses and induce fewer side effects.

### Limitations of allergen avoidance for airway allergies

Allergen avoidance is a valid strategy to reduce symptoms of food allergy, for example; however, it does not result in loss of allergy. Given the nature of the exposure, allergen avoidance is less efficient for allergic airway inflammation to inhaled antigens. IgE titers for certain (GC-dependent) allergens are long lasting; hence allergen avoidance would not reduce symptoms on re-exposure. For these allergens, immunotherapy to induce active tolerance would be a better choice. For weak allergens, such as house dust mite, allergen exposure can be reduced but not eliminated. Even if the IgE memory compartment would be inefficient or nonexistent, repeated or continuous low-level allergen exposure would ensure constant replenishment of allergen-specific $IgE^+$ B cells.

### IgE-targeting therapeutics

The importance of IgE in the pathology and symptoms of allergic airway disorders is substantiated by the effect of IgE-targeting biological agents on allergic asthma symptoms. Treatment of allergic diseases with humanized anti-IgE (omalizumab)

decreases serum IgE levels and prevents its interaction with mast cells and basophils. Lower serum IgE levels further decrease the expression of FcεRI on basophils and mast cells, thus rendering them less excitable. Repeated subcutaneous administration of omalizumab improves asthma symptoms and allows reduction of medication use.[78,79] It is worth noting that although omalizumab's effects are only modest and do not occur in all patients with atopic asthma, benefits of IgE blockade can be observed, even in the most severe cases.[80]

Since the success of omalizumab, other IgE-targeting strategies are being investigated. Among them is a peptide vaccine that elicits long-term protective autoantibodies against IgE. This would result in therapeutic effects similar to those of omalizumab without the need for frequent injections of recombinant antibody.[81] Others focus on the development of therapeutics targeting the proximal IgE-specific membrane sequence (constant domain of IgE, proximal [CεmX], or IgE membrane proximal domain [EMPD]) present in membrane IgE only. They developed antibodies against this unique sequence, which could target and eliminate IgE-committed and memory B cells.[82-84] The efficacy of these antibodies was shown by *in vitro* binding to mIgE transfectants and cell lines but will be difficult to prove in patients given the very low frequency of $IgE^+$ B cells in peripheral blood. Because binding of the antibody to mIgE provides BCR stimulation without cognate interaction with $CD4^+$ T cells, this results in receptor-mediated apoptosis. The mIgE antibody was thus able to block synthesis of IgE on allergen exposure in a murine model but did not affect established total IgE titers.[79] Therapies targeting long-lived PCs will be needed to reduce total IgE levels, but such therapies are not currently available.

## CONCLUSIONS

Although our knowledge is expanding, several important questions on the "who, where, and when" of IgE memory remain unclear. After careful review of the literature these are the up-to-date answers.

### "Who"

PCs are the most likely cellular source of IgE memory. They are readily detected both in animal models of allergic disease and in patients with increased serum IgE levels. Furthermore, their frequency correlates well with serum IgE levels. Detection of $mIgE^+$ memory B cells is technically difficult because of their extremely low frequencies. The little evidence on their existence remains to be confirmed.

### "Where"

Both human and animal studies show that the respiratory mucosa is a site of IgE induction during allergic airway inflammation. The role of airway draining lymph nodes in IgE induction needs to be revisited. IgE PCs are found in the bone marrow and spleen in animal models. Murine studies further show that the airway mucosa provides a survival niche for PCs through the production of prosurvival neurotrophins.

### "When"

GC dependence of the IgE response is still a subject of debate, but evidence from different animal models and patients suggests that $IgE^+$ B cells are dependent on GCs, although not in a conventional way, such as $IgG^+$ B cells. In some conditions, such as bacterial colonization, or more extreme situations, such as CD4 deficiency, IgE could arise in the absence of T-cell help.

## REFERENCES

1. Janeway C. Immunobiology: the immune system in health and disease. 6th ed. New York: Garland Science; 2005.
2. Maurer D, Stingl G. Immunoglobulin E-binding structures on antigen-presenting cells present in skin and blood. J Invest Dermatol 1995;104:707-10.
3. Maurer D, Fiebiger S, Ebner C, Reininger B, Fischer GF, Wichlas S, et al. Peripheral blood dendritic cells express Fc epsilon RI as a complex composed of Fc epsilon RI alpha- and Fc epsilon RI gamma-chains and can use this receptor for IgE-mediated allergen presentation. J Immunol 1996;157:607-16.
4. Novak N, Allam JP, Hagemann T, Jenneck C, Laffer S, Valenta R, et al. Characterization of FcepsilonRI-bearing CD123 blood dendritic cell antigen-2 plasmacytoid dendritic cells in atopic dermatitis. J Allergy Clin Immunol 2004;114:364-70.
5. Hammad H, Plantinga M, Deswarte K, Pouliot P, Willart MA, Kool M, et al. Inflammatory dendritic cells—not basophils—are necessary and sufficient for induction of Th2 immunity to inhaled house dust mite allergen. J Exp Med 2010;207:2097-111.
6. Gould HJ, Sutton BJ. IgE in allergy and asthma today. Nat Rev Immunol 2008;8:205-17.
7. McHeyzer-Williams LJ, McHeyzer-Williams MG. Antigen-specific memory B cell development. Annu Rev Immunol 2005;23:487-513.
8. Armitage RJ, Fanslow WC, Strockbine L, Sato TA, Clifford KN, Macduff BM, et al. Molecular and biological characterization of a murine ligand for CD40. Nature 1992;357:80-2.
9. Banchereau J, Bazan F, Blanchard D, Briere F, Galizzi JP, van Kooten C, et al. The CD40 antigen and its ligand. Annu Rev Immunol 1994;12:881-922.
10. Fazilleau N, Mark L, McHeyzer-Williams LJ, McHeyzer-Williams MG. Follicular helper T cells: lineage and location. Immunity 2009;30:324-35.
11. Dullaers M, Li D, Xue Y, Ni L, Gayet I, Morita R, et al. A T cell-dependent mechanism for the induction of human mucosal homing immunoglobulin A-secreting plasmablasts. Immunity 2009;30:120-9.
12. Linterman MA, Beaton L, Yu D, Ramiscal RR, Srivastava M, Hogan JJ, et al. IL-21 acts directly on B cells to regulate Bcl-6 expression and germinal center responses. J Exp Med 2009;207:353-63.
13. Zotos D, Coquet JM, Zhang Y, Light A, D'Costa K, Kallies A, et al. IL-21 regulates germinal center B cell differentiation and proliferation through a B cell-intrinsic mechanism. J Exp Med 2010;207:365-78.
14. Morita R, Schmitt N, Bentebibel SE, Ranganathan R, Bourdery L, Zurawski G, et al. Human blood CXCR5(+)CD4(+) T cells are counterparts of T follicular cells and contain specific subsets that differentially support antibody secretion. Immunity 2011;34:108-21.
15. Pene J, Rousset F, Briere F, Chretien I, Bonnefoy JY, Spits H, et al. IgE production by normal human lymphocytes is induced by interleukin 4 and suppressed by interferons gamma and alpha and prostaglandin E2. Proc Natl Acad Sci U S A 1988;85:6880-4.
16. Kawabe T, Naka T, Yoshida K, Tanaka T, Fujiwara H, Suematsu S, et al. The immune responses in CD40-deficient mice: impaired immunoglobulin class switching and germinal center formation. Immunity 1994;1:167-78.
17. Kuhn R, Rajewsky K, Muller W. Generation and analysis of interleukin-4 deficient mice. Science 1991;254:707-10.
18. Delphin S, Stavnezer J. Characterization of an interleukin 4 (IL-4) responsive region in the immunoglobulin heavy chain germline epsilon promoter: regulation by NF-IL-4, a C/EBP family member and NF-kappa B/p50. J Exp Med 1995;181:181-92.
19. Geha RS, Jabara HH, Brodeur SR. The regulation of immunoglobulin E class-switch recombination. Nat Rev Immunol 2003;3:721-32.
20. Tran TH, Nakata M, Suzuki K, Begum NA, Shinkura R, Fagarasan S, et al. B cell-specific and stimulation-responsive enhancers derepress Aicda by overcoming the effects of silencers. Nat Immunol 2010;11:148-54.
21. Muramatsu M, Sankaranand VS, Anant S, Sugai M, Kinoshita K, Davidson NO, et al. Specific expression of activation-induced cytidine deaminase (AID), a novel member of the RNA-editing deaminase family in germinal center B cells. J Biol Chem 1999;274:18470-6.
22. Cameron L, Gounni AS, Frenkiel S, Lavigne F, Vercelli D, Hamid Q. S epsilon S mu and S epsilon S gamma switch circles in human nasal mucosa following ex vivo allergen challenge: evidence for direct as well as sequential class switch recombination. J Immunol 2003;171:3816-22.
23. Takhar P, Smurthwaite L, Coker HA, Fear DJ, Banfield GK, Carr VA, et al. Allergen drives class switching to IgE in the nasal mucosa in allergic rhinitis. J Immunol 2005;174:5024-32.

24. Erazo A, Kutchukhidze N, Leung M, Christ AP, Urban JF Jr, Curotto de Lafaille MA, et al. Unique maturation program of the IgE response in vivo. Immunity 2007;26:191-203.
25. Radbruch A, Muehlinghaus G, Luger EO, Inamine A, Smith KG, Dorner T, et al. Competence and competition: the challenge of becoming a long-lived plasma cell. Nat Rev Immunol 2006;6:741-50.
26. Orgel HA, Hamburger RN, Bazaral M, Gorrin H, Groshong T, Lenoir M, et al. Development of IgE and allergy in infancy. J Allergy Clin Immunol 1975;56: 296-307.
27. Mitre E, Nutman TB. IgE memory: persistence of antigen-specific IgE responses years after treatment of human filarial infections. J Allergy Clin Immunol 2006; 117:939-45.
28. Steinberger P, Bohle B, di Padova F, Wrann M, Liehl E, Scheiner O, et al. Allergen-specific IgE production of committed B cells from allergic patients in vitro. J Allergy Clin Immunol 1995;96:209-18.
29. Niederberger V, Ring J, Rakoski J, Jager S, Spitzauer S, Valent P, et al. Antigens drive memory IgE responses in human allergy via the nasal mucosa. Int Arch Allergy Immunol 2007;142:133-44.
30. Takahama H, Furusawa S, Ovary Z. IgE memory B cells identified by the polymerase chain reaction. Cell Immunol 1997;176:34-40.
31. Manz RA, Arce S, Cassese G, Hauser AE, Hiepe F, Radbruch A. Humoral immunity and long-lived plasma cells. Curr Opin Immunol 2002;14:517-21.
32. Slifka MK, Antia R, Whitmire JK, Ahmed R. Humoral immunity due to long-lived plasma cells. Immunity 1998;8:363-72.
33. Bellou A, Kanny G, Fremont S, Moneret-Vautrin DA. Transfer of atopy following bone marrow transplantation. Ann Allergy Asthma Immunol 1997;78:513-6.
34. Luger EO, Fokuhl V, Wegmann M, Abram M, Tillack K, Achatz G, et al. Induction of long-lived allergen-specific plasma cells by mucosal allergen challenge. J Allergy Clin Immunol 2009;124:819-26, e4.
35. Ganzer U, Bachert C. Localization of IgE synthesis in immediate-type allergy of the upper respiratory tract. ORL J Otorhinolaryngol Relat Spec 1988;50:257-64.
36. McMenamin C, Girn B, Holt PG. The distribution of IgE plasma cells in lymphoid and non-lymphoid tissues of high-IgE responder rats: differential localization of antigen-specific and "bystander" components of the IgE response to inhaled antigen. Immunology 1992;77:592-6.
37. Huggins KG, Brostoff J. Local production of specific IgE antibodies in allergic-rhinitis patients with negative skin tests. Lancet 1975;2:148-50.
38. Merrett TG, Houri M, Mayer AL, Merrett J. Measurement of specific IgE antibodies in nasal secretion—evidence for local production. Clin Allergy 1976;6:69-73.
39. Diaz-Sanchez D. The role of diesel exhaust particles and their associated polyaromatic hydrocarbons in the induction of allergic airway disease. Allergy 1997;52: 52-8.
40. Durham SR, Gould HJ, Thienes CP, Jacobson MR, Masuyama K, Rak S, et al. Expression of epsilon germ-line gene transcripts and mRNA for the epsilon heavy chain of IgE in nasal B cells and the effects of topical corticosteroid. Eur J Immunol 1997;27:2899-906.
41. KleinJan A, Vinke JG, Severijnen LW, Fokkens WJ. Local production and detection of (specific) IgE in nasal B-cells and plasma cells of allergic rhinitis patients. Eur Respir J 2000;15:491-7.
42. Smurthwaite L, Walker SN, Wilson DR, Birch DS, Merrett TG, Durham SR, et al. Persistent IgE synthesis in the nasal mucosa of hay fever patients. Eur J Immunol 2001;31:3422-31.
43. Bachert C, Gevaert P, Holtappels G, Johansson SG, van Cauwenberge P. Total and specific IgE in nasal polyps is related to local eosinophilic inflammation. J Allergy Clin Immunol 2001;107:607-14.
44. Van Zele T, Gevaert P, Holtappels G, van Cauwenberge P, Bachert C. Local immunoglobulin production in nasal polyposis is modulated by superantigens. Clin Exp Allergy 2007;37:1840-7.
45. Ying S, Humbert M, Meng Q, Pfister R, Menz G, Gould HJ, et al. Local expression of epsilon germline gene transcripts and RNA for the epsilon heavy chain of IgE in the bronchial mucosa in atopic and nonatopic asthma. J Allergy Clin Immunol 2001;107:686-92.
46. Scanlon KM, Hawksworth RJ, Lane SJ, Mahon BP. IL-17A induces CCL28, supporting the chemotaxis of IgE-secreting B cells. Int Arch Allergy Immunol 2011; 156:51-61.
47. Lambrecht BN, De Veerman M, Coyle AJ, Gutierrez-Ramos JC, Thielemans K, Pauwels RA. Myeloid dendritic cells induce Th2 responses to inhaled antigen, leading to eosinophilic airway inflammation. J Clin Invest 2000;106:551-9.
48. Gould HJ, Takhar P, Harries HE, Durham SR, Corrigan CJ. Germinal-centre reactions in allergic inflammation. Trends Immunol 2006;27:446-52.
49. Cameron LA, Durham SR, Jacobson MR, Masuyama K, Juliusson S, Gould HJ, et al. Expression of IL-4, Cepsilon RNA, and Iepsilon RNA in the nasal mucosa of patients with seasonal rhinitis: effect of topical corticosteroids. J Allergy Clin Immunol 1998;101:330-6.
50. Humbert M, Durham SR, Kimmitt P, Powell N, Assoufi B, Pfister R, et al. Elevated expression of messenger ribonucleic acid encoding IL-13 in the bronchial mucosa of atopic and nonatopic subjects with asthma. J Allergy Clin Immunol 1997;99:657-65.
51. Gauchat JF, Henchoz S, Mazzei G, Aubry JP, Brunner T, Blasey H, et al. Induction of human IgE synthesis in B cells by mast cells and basophils. Nature 1993; 365:340-3.
52. Cameron L, Hamid Q, Wright E, Nakamura Y, Christodoulopoulos P, Muro S, et al. Local synthesis of epsilon germline gene transcripts, IL-4, and IL-13 in allergic nasal mucosa after ex vivo allergen exposure. J Allergy Clin Immunol 2000;106:46-52.
53. Takhar P, Corrigan CJ, Smurthwaite L, O'Connor BJ, Durham SR, Lee TH, et al. Class switch recombination to IgE in the bronchial mucosa of atopic and nonatopic patients with asthma. J Allergy Clin Immunol 2007;119:213-8.
54. Hahn C, Islamian AP, Renz H, Nockher WA. Airway epithelial cells produce neurotrophins and promote the survival of eosinophils during allergic airway inflammation. J Allergy Clin Immunol 2006;117:787-94.
55. Abram M, Wegmann M, Fokuhl V, Sonar S, Luger EO, Kerzel S, et al. Nerve growth factor and neurotrophin-3 mediate survival of pulmonary plasma cells during the allergic airway inflammation. J Immunol 2009;182:4705-12.
56. Quarcoo D, Schulte-Herbruggen O, Lommatzsch M, Schierhorn K, Hoyle GW, Renz H, et al. Nerve growth factor induces increased airway inflammation via a neuropeptide-dependent mechanism in a transgenic animal model of allergic airway inflammation. Clin Exp Allergy 2004;34:1146-51.
57. Durkin HG, Chice SM, Gaetjens E, Bazin H, Tarcsay L, Dukor P. Origin and fate of IgE-bearing lymphocytes. II. Modulation of IgE isotype expression on Peyer's patch cells by feeding with certain bacteria and bacterial cell wall components or by thymectomy. J Immunol 1989;143:1777-83.
58. Wang CH, Richards EM, Block RD, Lezcano EM, Gutierrez R. Early induction and augmentation of parasitic antigen-specific antibody-producing B lymphocytes in the non-Peyer's patch region of the small intestine. Front Biosci 1998;3:A58-65.
59. Kelly KA, Butch AW. Antigen-specific immunoglobulin E+ B cells are preferentially localized within germinal centres. Immunology 2007;120:345-53.
60. Auci DL, Chice SM, Heusser C, Athanassiades TJ, Durkin HG. Peyer's patches and mesenteric lymph nodes are the sites of first appearance of IgE bearing B lymphocytes and hapten specific IgE antibody forming cells in BPO-KLH sensitized mice. Reg Immunol 1992;4:216-24.
61. Aalberse RC, Platts-Mills TA. How do we avoid developing allergy: modifications of the TH2 response from a B-cell perspective. J Allergy Clin Immunol 2004;113: 983-6.
62. Payet-Jamroz M, Helm SL, Wu J, Kilmon M, Fakher M, Basalp A, et al. Suppression of IgE responses in CD23-transgenic animals is due to expression of CD23 on nonlymphoid cells. J Immunol 2001;166:4863-9.
63. Platts-Mills T, Vaughan J, Squillace S, Woodfolk J, Sporik R. Sensitisation, asthma, and a modified Th2 response in children exposed to cat allergen: a population-based cross-sectional study. Lancet 2001;357:752-6.
64. Dahlke I, Nott DJ, Ruhno J, Sewell WA, Collins AM. Antigen selection in the IgE response of allergic and nonallergic individuals. J Allergy Clin Immunol 2006; 117:1477-83.
65. Snow RE, Djukanovic R, Stevenson FK. Analysis of immunoglobulin E VH transcripts in a bronchial biopsy of an asthmatic patient confirms bias towards VH5, and indicates local clonal expansion, somatic mutation and isotype switch events. Immunology 1999;98:646-51.
66. Coker HA, Durham SR, Gould HJ. Local somatic hypermutation and class switch recombination in the nasal mucosa of allergic rhinitis patients. J Immunol 2003; 171:5602-10.
67. Snow RE, Chapman CJ, Frew AJ, Holgate ST, Stevenson FK. Pattern of usage and somatic hypermutation in the V(H)5 gene segments of a patient with asthma: implications for IgE. Eur J Immunol 1997;27:162-70.
68. Snow RE, Chapman CJ, Holgate ST, Stevenson FK. Clonally related IgE and IgG4 transcripts in blood lymphocytes of patients with asthma reveal differing patterns of somatic mutation. Eur J Immunol 1998;28:3354-61.
69. Coker HA, Harries HE, Banfield GK, Carr VA, Durham SR, Chevretton E, et al. Biased use of VH5 IgE-positive B cells in the nasal mucosa in allergic rhinitis. J Allergy Clin Immunol 2005;116:445-52.
70. Kluytmans J, van Belkum A, Verbrugh H. Nasal carriage of *Staphylococcus aureus*: epidemiology, underlying mechanisms, and associated risks. Clin Microbiol Rev 1997;10:505-20.
71. Riechelmann H, Essig A, Deutschle T, Rau A, Rothermel B, Weschta M. Nasal carriage of Staphylococcus aureus in house dust mite allergic patients and healthy controls. Allergy 2005;60:1418-23.
72. Gevaert P, Holtappels G, Johansson SG, Cuvelier C, Cauwenberge P, Bachert C. Organization of secondary lymphoid tissue and local IgE formation to Staphylococcus aureus enterotoxins in nasal polyp tissue. Allergy 2005;60:71-9.

73. McCoy KD, Harris NL, Diener P, Hatak S, Odermatt B, Hangartner L, et al. Natural IgE production in the absence of MHC Class II cognate help. Immunity 2006;24:329-39.
74. Felices M, Yin CC, Kosaka Y, Kang J, Berg LJ. Tec kinase Itk in gammadeltaT cells is pivotal for controlling IgE production in vivo. Proc Natl Acad Sci U S A 2009;106:8308-13.
75. Chen K, Xu W, Wilson M, He B, Miller NW, Bengten E, et al. Immunoglobulin D enhances immune surveillance by activating antimicrobial, proinflammatory and B cell-stimulating programs in basophils. Nat Immunol 2009;10:889-98.
76. Finkelman FD, Snapper CM, Mountz JD, Katona IM. Polyclonal activation of the murine immune system by a goat antibody to mouse IgD. IX. Induction of a polyclonal IgE response. J Immunol 1987;138:2826-30.
77. Finkelman FD, Katona IM, Urban JF Jr, Holmes J, Ohara J, Tung AS, et al. IL-4 is required to generate and sustain in vivo IgE responses. J Immunol 1988;141: 2335-41.
78. Busse W, Corren J, Lanier BQ, McAlary M, Fowler-Taylor A, Cioppa GD, et al. Omalizumab, anti-IgE recombinant humanized monoclonal antibody, for the treatment of severe allergic asthma. J Allergy Clin Immunol 2001;108:184-90.
79. Milgrom H, Berger W, Nayak A, Gupta N, Pollard S, McAlary M, et al. Treatment of childhood asthma with anti-immunoglobulin E antibody (omalizumab). Pediatrics 2001;108:E36.
80. Avila PC. Does anti-IgE therapy help in asthma? Efficacy and controversies. Annu Rev Med 2007;58:185-203.
81. Peng Z, Liu Q, Wang Q, Rector E, Ma Y, Warrington R. Novel IgE peptide-based vaccine prevents the increase of IgE and down-regulates elevated IgE in rodents. Clin Exp Allergy 2007;37:1040-8.
82. Chen JB, Wu PC, Hung AF, Chu CY, Tsai TF, Yu HM, et al. Unique epitopes on C epsilon mX in IgE-B cell receptors are potentially applicable for targeting IgE-committed B cells. J Immunol 2010;184:1748-56.
83. Feichtner S, Infuhr D, Achatz-Straussberger G, Schmid D, Karnowski A, Lamers M, et al. Targeting the extracellular membrane-proximal domain of membrane-bound IgE by passive immunization blocks IgE synthesis in vivo. J Immunol 2008;180:5499-505.
84. Brightbill HD, Jeet S, Lin Z, Yan D, Zhou M, Tan M, et al. Antibodies specific for a segment of human membrane IgE deplete IgE-producing B cells in humanized mice. J Clin Invest 2010;120:2218-29.
85. Burrows B, Martinez FD, Halonen M, Barbee RA, Cline MG. Association of asthma with serum IgE levels and skin-test reactivity to allergens. N Engl J Med 1989;320:271-7.
86. Jungsuwadee P, Benkovszky M, Dekan G, Stingl G, Epstein MM. Repeated aerosol allergen exposure suppresses inflammation in B-cell-deficient mice with established allergic asthma. Int Arch Allergy Immunol 2004;133:40-8.
87. Korsgren M, Erjefalt JS, Korsgren O, Sundler F, Persson CG. Allergic eosinophil-rich inflammation develops in lungs and airways of B cell-deficient mice. J Exp Med 1997;185:885-92.
88. Perkins C, Wills-Karp M, Finkelman FD. IL-4 induces IL-13-independent allergic airway inflammation. J Allergy Clin Immunol 2006;118:410-9.
89. Tanabe T, Fujimoto K, Yasuo M, Tsushima K, Yoshida K, Ise H, et al. Modulation of mucus production by interleukin-13 receptor alpha2 in the human airway epithelium. Clin Exp Allergy 2008;38:122-34.
90. Maurer D, Fiebiger E, Reininger B, Ebner C, Petzelbauer P, Shi GP, et al. Fce receptor I on dendritic cells delivers IgE-bound multivalen antigens into a cathepsin S-dependent pathway of MHC class II presentation. J Immunol 1998; 161:2731-9.
91. Karagiannis SN, Warrack JK, Jennings KH, Murdock PR, Christie G, Moulder K, et al. Endocytosis and recycling of the complex between CD23 and HLA-DR in human B cells. Immunology 2001;103:319-31.
92. Tu Y, Salim S, Bourgeois J, Di Leo V, Irvine EJ, Marshall JK, et al. CD23-mediated IgE transport across human intestinal epithelium: inhibition by blocking sites of translation or binding. Gastroenterology 2005;129:928-40.
93. Hakonarson H, Carter C, Kim C, Grunstein MM. Altered expression and action of the low-affinity IgE receptor FcepsilonRII (CD23) in asthmatic airway smooth muscle. J Allergy Clin Immunol 1999;104:575-84.
94. Yamauchi K, Inoue H. Airway remodeling in asthma and irreversible airflow limitation-ECM deposition in airway and possible therapy for remodeling. Allergol Int 2007;56:321-9.
95. Abbas AK, Lichtman AH. Cellular and molecular immunology. 5th ed. Philadelphia: Saunders; 2005.
96. Karnowski A, Achatz-Straussberger G, Klockenbusch C, Achatz G, Lamers MC. Inefficient processing of mRNA for the membrane form of IgE is a genetic mechanism to limit recruitment of IgE-secreting cells. Eur J Immunol 2006;36: 1917-25.
97. Luger E, Lamers M, Achatz-Straussberger G, Geisberger R, Infuhr D, Breitenbach M, et al. Somatic diversity of the immunoglobulin repertoire is controlled in an isotype-specific manner. Eur J Immunol 2001;31:2319-30.
98. Donohoe PJ, Heddle RJ, Sykes PJ, Fusco M, Flego LR, Zola H. IgE+ cells in the peripheral blood of atopic, nonatopic, and bee venom-hypersensitive individuals exhibit the phenotype of highly differentiated B cells. J Allergy Clin Immunol 1995;95:587-96.
99. Stein LD, Chan MA, Hibi T, Dosch HM. Epstein-Barr virus-induced IgE production in limiting dilution cultures of normal human B cells. Eur J Immunol 1986; 16:1167-70.
100. MacKenzie T, Dosch HM. Clonal and molecular characteristics of the human IgE-committed B cell subset. J Exp Med 1989;169:407-30.
101. Dhanjal MK, Towler AE, Tuft S, Hetzel C, Richards D, Kemeny DM. The detection of IgE-secreting cells in the peripheral blood of patients with atopic dermatitis. J Allergy Clin Immunol 1992;89:895-904.
102. King CL, Poindexter RW, Ragunathan J, Fleisher TA, Ottesen EA, Nutman TB. Frequency analysis of IgE-secreting B lymphocytes in persons with normal or elevated serum IgE levels. J Immunol 1991;146:1478-83.
103. Horst A, Hunzelmann N, Arce S, Herber M, Manz RA, Radbruch A, et al. Detection and characterization of plasma cells in peripheral blood: correlation of IgE+ plasma cell frequency with IgE serum titre. Clin Exp Immunol 2002; 130:370-8.