

REVIEW

Immunogenetic characteristics of immunoglobulin E in allergic disease

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Summary

Patients with allergic diseases produce an excess of allergen-specific IgE, the specific effector molecule that triggers allergic reactions. The provocation for this excess IgE production is still uncertain. Current ideas include oligoclonal expansion of allergen-specific B cells emanating from germinal centres, activation by superantigen of a subset of B cells, or polyclonal B cells class switching to IgE due to an IL-4 predominance. Additionally, genetic elements contribute to a propensity for increased allergen-specific IgE production. The procedure of RT-PCR allows for amplification of infrequent IgE mRNA transcripts from B cells of atopic individuals, and so facilitates examination of expressed Ig cDNA sequences. Better knowledge of the molecular characteristics of IgE produced by patients with allergic diseases would elucidate the immunogenetic basis for elevated allergen-specific IgE levels. The 'immunogenetic footprint' of IgE transcripts may elucidate the origin and activation of IgE-producing B cells in allergic disease. Here we review studies of the immunogenetic features of IgE in allergic diseases, highlighting the major advances and the experimental limitations.

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Quantitative aspects of immunoglobulin E

Allergic diseases, including allergic rhinitis (AR), asthma and atopic dermatitis, have increased in prevalence in the developed world over recent decades and this has been paralleled by increasing levels of serum-specific IgE to common environmental allergens [1]. Under normal circumstances, the concentration of IgE in serum is four orders of magnitude lower than other classes of Ig. Correspondingly, the frequency of IgE-producing B cells is 0.4–2 per 10^5 circulating B cells vs. 80% of B cells that produce IgM or 16% that produce IgG [2]. Recruitment of IgE-committed cells is in part restricted by low B cell receptor expression due to the presence of multiple and deviant 3' polyadenylation signals on the mRNA for membrane IgE, and this low expression leads to a diminished capacity for B cell receptor engagement [3]. However, on effector cells, the affinity of FcεRI for IgE, 10^{-10} M, is 100-fold higher than the affinity of the Fcγ RI for IgG, 10^{-8} M. Thus, despite serum IgE levels being in the nanogram per millilitre concentration range, a low percentage of peripheral B cells expressing IgE, with a decreased capacity for activation, IgE bound by effector cells has a long half life. The high affinity of the FcεRI for IgE can be explained structurally by a conformational

difference between the two epsilon 4 constant domains (CE4) induced upon binding of IgE [4]. This conformational change effectively locks the IgE onto its receptor and retards dissociation of the IgE–receptor complex [5]. Therefore, small amounts of IgE may have profound physiological effects.

Molecular mechanisms for generation of antibody diversity

An understanding of the forces driving IgE responses in allergic diseases requires appreciation of the mechanisms underlying the molecular diversity in the antibody repertoire [6, 7]. The dissection of the molecular machinery responsible for V(D)J recombination in developing B cells in bone marrow and class switch recombination (CSR) and somatic hypermutation (SHM) in activated peripheral B cells are well researched [8, 9]. The SHM of Ig variable regions of B cells in lymphoid germinal centres during an adaptive immune response allows for selection of B cells that can produce antibody with increased affinity for antigen. Notably, SHM does not occur randomly throughout the variable domains of the Ig genes, but rather is focused at mutational hot spots with a DNA sequence motif of RGYW, where R is an adenine (A)

or guanine (G), Y is a cytidine (C) or thymidine (T) and W is an A or T [10, 11]. In affinity-matured B cells, SHM is observed to cluster in the antigen complementarity determining regions (CDR) of the variable (V) domains. The clustering of SHM is attributed to antigenic selection of B cells expressing antibody with improved affinity for antigen facilitated by competition for antigen presented by follicular dendritic cells in the germinal centre [12]. The process of antigenic selection of antibodies with high affinity may mask an underlying bias for SHM to occur closer to the promotor because the enzymes responsible for introducing double-strand breaks in the Ig gene require access to the DNA provided during transcription [13]. Thus, the rate of SHM decays exponentially with distance from the promotor, restricting SHM to the V regions of Ig genes [14]. Amino acid constraints and structural stability of the resulting antibody are also important selective pressures on the SHM of Ig genes [15–17].

Peripheral receptor revision of expressed Ig genes is now believed to occur. Mature B cells expressing recombined antibody can undergo further Ig gene recombination [18, 19]. V–D–J recombination is a process occurring centrally in the bone marrow where recombination activation genes (RAG) are expressed in developing B cells, whereas later stage changes to Ig genes including SHM and consequent antigen selection are processes occurring peripherally in germinal centres. Evidence of peripheral expression of RAG under certain conditions has provided an explanation for peripheral receptor revision, primarily described in relation to B cell tolerance in autoimmunity [18, 19]. This process could also contribute to IgE diversity noting that RAG-1 and RAG-2 expression can be induced by CD40L, Blys and APRIL in combination with IL-4 but not IL-13, conditions that may favour IgE-producing B cells [20].

The heavy chain complementarity determining region 3 diversity: importance for antibody specificity

The CDR3 loop of the heavy chain (HC) is accepted as the region of an antibody with the greatest sequence diversity and capacity for structural variation. The CDR 1, 2 and 3 loops of the light chain and CDR 1 and 2 loops of the HC have a limited range of structurally permissible sizes and conformations, whereas the HC CDR3 can accommodate a larger number of structural possibilities [21, 22]. The HC CDR3 encompasses the junction of the variable domain of the heavy chain (VH)–D–JH genes and has the greatest number of permutations of possible VH, D and JH genes within the one small region of an antibody combining site. Also the activity of DNA polymerases, exonucleases and terminal deoxynucleotidyl transferase that introduce non-encoded (N) nucleotides

and nucleotides palindromic (P) to the double-strand DNA splice site at these junctions creates further diversity in the HC CDR3 [23]. There is also the possibility of inclusion of more than one D gene by the processes of D–D gene fusion and insertion of nucleotides palindromic to the D gene [24]. IgE sequences have been observed to include more than one D gene segment [25, 26]. The molecular mechanisms are controversial and in fact many apparent D–D fusions are likely to be the result of terminal deoxynucleotidyl transferase nucleotide insertion [27]. It is often difficult to assign which molecular processes have contributed to the nucleotides in the VH–D–JH junctions.

A challenge for the analysis of the relative roles of junctional diversity and SHM at the CDR3 of the HC is the capacity to assign the correct germline D gene encoding the loop. Publicly available algorithms such as VQUEST at the ImMunoGeneTics (IMGT) Ig database frequently fail to generate meaningful alignments of expressed Ig sequences with germline D genes [28]. Without accurately assigned D genes, the role of various molecular mechanisms responsible for the origin of a given Ig sequence can be problematic and interpretations of the underlying cellular interactions unreliable [25, 29].

The high degree of structural and sequence variability in the HC CDR3 makes it the most diverse region of any given antibody and confers a great degree of specificity upon the antibody [30]. Hence, many molecular immunologists have limited their research to the Ig HC, although the contribution of the light chain is still important. Analysis of the pattern of SHM and characteristics of the HC CDR3 of IgE from atopic individuals are highly informative for whether particular VH–CE gene transcripts are derived from antigen-driven affinity-matured B cell responses, activation of B cells via superantigen interaction with framework region (FWR) of antibodies or a generalized increase in antibody class switching to IgE due to the T-helper 2 type cytokine environment that prevails in response to allergens. For this reason a number of studies have investigated the immunogenetic characteristics of IgE HC transcripts from patients with allergic diseases with the aim of inferring the *in vivo* origin of the B cells producing them.

Variable domain of the heavy chain gene usage in immunoglobulin E transcripts of patients with allergic diseases

Several studies have shown that patients with asthma preferentially utilize the minor VH5 gene family for VH–CE transcripts but not other HC VH–JH gene rearrangements in B cells from peripheral blood mononuclear cells (PBMC), spleen and/or bronchial sources (Table 1)

Table 1. Studies of VH gene usage in IgE responses of allergic individuals

Allergic disease	Allergen	Subjects (number)	Source	VH-CE transcripts/ subjects	VH gene usage	Reference
Atopic dermatitis, asthma	HDM	3	PBMC	8, 7, 4	VH3, 4, 5 and 6 amplified, VH5 cloned; VH5 clones had FWR mutations inconsistent with Ag selection	[31]
Asthma	HDM	1	Spleen	14	4/14 were VH5, mutational hot spots throughout	[10]
Asthma	HDM	8	PBMC	7–17	VH5 biased, FWR mutations	[32–34]
Asthma	HDM, GP	1	PBMC	10	VH5 amplified and cloned for IgE, IgM and IgG; mutational hot spots, e.g. Ser72 and Lys74 in FWR	
Asthma	HDM	2		9, 20	Various genes, five and nine unique clones, respectively, increase CSR to IgE due to Th2 cytokine profile	[35]
Asthma	HDM, cat, tree, GP	4	PBMC	Seven VH-CE, and IgG4	Clonally related IgE and IgG4 clones sharing SHM in three of four patients	[36]
AR	GP	1	PBMC	4	IgE Fab expression library from which four Phl p 5-specific FAb with same HC paired with different light chains selected	[37]
Peanut allergy	Peanuts	2	PBMC	Five of 26 unique; 12 of 21 unique	Increased use of VH1 family but various genes; 1.02, 1.46 and 1.18 and 1.69 and 1.8, SHM in CDR	[38]
Asthma	HDM	1	Bronchial mucosa	30	10/30 unique clones utilized VH5 family; three unmutated IgE sequences, clonally related IgM, IgG and IgE	[34]
Food allergy	OA	1	PBMC	51	VH6 in 33%, low frequency of SHM, short CDR3	[39]
AR	Multiple	6	Nasal mucosa	15 ea.	Clonally related families of IgE, IgE related to IgA, no IgE from non-atopic mucosa, three/six with biased VH5 use	[40]
AR, asthma	GP, HDM	3	PBMC	20	Multiple related clones, repeated use of particular genes, e.g. VH3–9, 3–11, VH1–69, VH5a, SHM in CDR, high frequency of SHM	[41]
Food allergy	CM, egg	3	PBMC	8, 13, 15 (children)	Expansion of related clones, SHM in CDR and FWR, related VH1–69 clones with same CDR3 loop in three patients, two patients with VH3–11 usage	[42]
AR	Multiple	11	Nasal mucosa	62 in total	Biases VH5 use in five of 11 patients, one patient with 11 unrelated VH5 clones, SHM in FWR and CDR, mutational hot spots, no IgE from control samples	[43]
AR	GP, cat, tree and mugwort pollen	2	PBMC	63 and 51	Broad Ig gene usage consistent with peripheral repertoire, location of SHM not shown. ScFv library generated and iPhl p 5-specific scFv isolated, which were derived from multiple different clones and were patient specific	[44]
Atopic dermatitis	HDM, animal dander, pollens	14	PBMC	FWR3 and CDR3 of VH family 3, three patients only	VH family usage in IgE transcripts similar to IgM and IgG, no common VH bias between patients, broad range of CDR3 spectra, sequences for one patient showed 54/58 transcripts used VH3–73, only 46% showed signs of SHM, FWR3 mutations in three patients ranged from 3–25% per 100 bp	[45]

OA, ovalbumin; HDM, house dust mite; CM, cow's milk; GP, grass pollen allergens including Timothy, canary, Bermuda and ryegrass; VH, variable domain of the heavy chain; CE, epsilon constant domain; AR, allergic rhinitis; PBMC, peripheral blood mononuclear cells; FWR, frame work region; Ag, antigen; CSR, class switch recombination; Th2, T-helper type 2; SHM, somatic hypermutation; CDR, complementarity determining regions; scFv, single chain variable fragment.

[26, 32–34, 36]. Similar overuse of VH5 in IgE transcripts from nasal biopsies of patients with AR has also been observed [40, 43, 46]. Preference for the VH5 gene segment in IgE responses of multiple patients suggests a superantigen-like activation of these B cells [33, 47]. Examination of the VH5 IgE transcripts revealed mutational hot spots, for example Ser28, Ser72 and Lys74, within the FWR, which indicated non-paratope interaction between allergen and FWR of the antibody, consistent with superantigen activation [33]. Some of the mutational hot spots were located in or near the junctions of the CDR

(Ser28 and Ser31) and could form part of a traditional allergen–antibody interface for these VH5 antibodies. The Chothia and Kabat Ig amino acid numbering systems vary at this region of the antibody, providing some discrepancy for the assignation of the junction of this CDR1 loop and the FWR [21]. Crystallization studies of antibody domains encoded by VH5 have not been conducted and the light chains utilized are not known, but a model of an antibody encoded by VH5–51 showing the location of non-intrinsic mutational hot spots is consistent with the superantigen hypothesis [43]. The source of superantigen could be

house dust mite (HDM) allergen to which the patients in these studies were sensitive [26, 32–34, 36]. However, the observed major histocompatibility complex class II restriction specificity of human T cell recognition of HDM antigen is not consistent with a superantigen within HDM extracts, at least in regard to T cell activation [48, 49]. An alternative explanation, which cannot be tested without structural studies, is that these antibodies bind antigen at the paratope but amino acid mutations accumulated in the FWR alter the shape of the antigen interface encoded by CDR, without being directly involved in antigen binding themselves. The increase in SHM in FWR may be because of an overaccumulation of mutations throughout the VH region due to the excessive chronic stimulation of the IgE-producing B cells by the perennial exposure to HDM allergens. Another explanation is that some patients with chronic airway inflammation harbour persistent infections that could provide a source of superantigen for interaction with VH5 family IgE rather than allergen, although this is not proven.

Van der Stoep et al. [31] report the analysis of VH5 family IgE transcripts from the peripheral blood of three patients with atopic dermatitis. They observed both repeated use of VH5 IgE transcripts with distinct CDR3 as well as related clones isolated from the same patient. The high frequency and location of replacement mutations in the FWR of the VH5 transcripts suggested chronic antigenic exposure, although the clinical sensitivities of the patients were not given. The mutational hot spots previously observed in patients with asthma were present in the VH5 IgE transcripts from these patients with atopic dermatitis. This may also suggest that chronic exposure to bacterial antigens is responsible for stimulating these VH5-encoding IgE transcripts because infection is a common complication in atopic dermatitis. This hypothesis warrants follow-up because evidence for bacterial provocation of IgE synthesis in atopic diseases would alter treatment strategies.

Another group found a lack of clonally related CDR3 sequences among VH–CE transcripts amplified from peripheral blood B cells from two subjects with atopic dermatitis and HDM allergy (Table 1) [35]. Although nine and 20 transcripts were sampled for each patient, only five and seven, respectively, were unique for each subject. The common VH3–23 and VH3–30 were among the VH gene segments utilized and it was concluded that there was a generalized increase in class switching to IgE due to the atopic condition. However, identical transcripts were repeatedly isolated without closely related sequences to suggest an expansion of B cell clones *in vivo*. It is possible that the initial pool of B cells was so small that repeated occurrence of the same sequence may be an amplification effect in this instance.

A study of two subjects allergic to peanut allergens reported preferential usage of the VH1 gene family in

VH–CE transcripts (Table 1) [38]. Initial analysis of these data supports the hypothesis that an IgE antibody response to a given antigen utilizes a particular VH gene family, but for one subject 12 of 21 transcripts and for the second subject five of 26 transcripts examined were unique. Among these transcripts there was a broad usage of different VH genes within VH family 1, suggestive of an oligoclonal expansion of B cells. The clustering of SHM to the CDR3 was also consistent with antigen-driven B cell responses.

Eibensteiner et al. [50] examined the VH–CE gene usage of 10 atopic individuals with IgE reactivity to a number of clinically important allergens, including domestic pet, fungus, mite, tree and weed pollen allergens. Amplified VH–CE products were probed for each of the VH families by Southern blotting, and blots for most of the subjects had signals for each of the VH gene families, yet there were marked differences in intensities between VH families. Southern blotting is not quantitative but the data showed a broad VH gene usage in IgE responses of atopic individuals. The advantage of this approach is that a larger number of subjects were analysed, but it did not allow for examination of usage of particular Ig genes within VH families, clonal relatedness, CDR composition nor patterns of SHM.

A study of a phage-displayed IgE antibody fragment (FAB) library derived from peripheral blood B cells of a patient with atopic dermatitis and egg allergy supports the importance of the HC CDR3 in antibody reactivity. Screening of this library with ovomucoid yielded 12 IgE FAB clones that used the VH6.01 HC (Table 1) [39]. Even in the unselected IgE FAB library there was disproportional overrepresentation of the VH6 gene. A number of the selected clones were polyreactive, recognizing the major egg allergen ovomucoid as well as human IgG, tetanus toxoid and von Willebrand's factor. Cyclic synthetic peptides based on the HC CDR3 loop sequence of three of these IgE FAB bound the same antigens and inhibited the binding of the expressed IgE FABs, indicating that specificity was conferred by the HC CDR3. It is possible that the polyreactivity of the ovomucoid-selected FAB was due to a lack of specificity conferred by the light chain(s) naturally paired with the VH6.01 HC *in vivo*. The low level of SHM throughout the VH region and short CDR3 length, without non-encoded nucleotide additions by terminal deoxynucleotidyl transferase, seemed more suggestive of B-1 B cells than of antigen-driven affinity-matured antibody responses of the adaptive antibody repertoire that originate from the B-2 B cells in germinal centres [39]. This report is the first suggestion of polyspecific IgE responses with characteristics of B-1-like B cells [51], as discussed below.

In a similar study, Steinberger et al. [37] selected timothy grass allergen Phl p 5-specific IgE FAB from a phage-displayed IgE library in which four clones utilized

the same HC paired with different light chains. In a follow-up study the single chain variable fragment derived from the expressed IgE repertoire were created to select clones reactive with Phl p 5 [44]. Andreasson et al. [44] concluded that the IgE repertoire of two seasonal rhinitis patients with sensitivities to several allergens including timothy grass pollen was consistent with a typical peripheral B cell population, but the locations of SHM throughout the V genes were not analysed.

We examined VH gene characteristics of IgE transcripts in three grass pollen allergic subjects with seasonal rhinitis (Table 1) [41]. Each subject had a unique VH gene usage pattern in the 20 IgE transcripts sampled, and there was evidence of both clonal relatedness among the transcripts and repeated usage of the same VH genes by a number of distinct clones. The level of homology with the closest germline gene in most transcripts ranged from 86% to 94%, the distribution of SHM was primarily in the CDR regions, and the clonal relationships among the IgE transcripts were all consistent with oligoclonal expansion of an antigen-driven B cell response. However, one subject had a number of IgE transcripts of the uncommon VH3-9 and VH3-11 gene segments with a low level of somatic mutations more suggestive of B-1-like B cell responses, because of both these features. Interestingly, for one of the subjects with AR and asthma who was also sensitive to HDM, 30% of the IgE transcripts were of the VH5 family. As a control, we amplified and cloned the IgM transcripts from one of the subjects; in contrast to the IgE transcripts, not one IgM clone utilized the same VH-D-JH combinations and the VH genes were all 99–100% homologous to their germline VH gene [41]. These data did not support a generalized increase in CSR to IgE, nor superantigen activation of B cells subsets, but rather suggested oligoclonal expansion of antigen-driven B cell responses.

A recent study of 14 patients with atopic dermatitis, and with sensitivities to multiple allergens, found that the VH gene family usage in IgE transcripts was similar to those of IgM and IgG, with the common VH3 and 4 families predominating [45]. The CDR3 spectra of IgE transcripts were examined to demonstrate a broad IgE repertoire in these patients. The FWR3 and CDR3 of VH3 family IgE transcripts were cloned and sequenced for three patients (data accessed www.jacionline.org). Remarkably, one patient had 54 out of 58 VH family 3-CE transcripts encoded by VH3-73. There was repeated use of this VH gene in all three patients examined with both clonally related and distinct CDR3, which indicates that there may be a misassignment of VH gene or a primer bias because VH3.73 is not known to be commonly utilized in the adult PBMC repertoire [52]. It was reported that 48% of these genes were somatically mutated with mutations in FWR3 ranging from 3% to 25% per 100 bp, which is unusually high. The alignment between these IgE sequences with the closest germline Ig gene segment was not shown. Lim et al.

also describe the changes in the CDR3 spectra of IgE, IgM and IgG transcripts in three patients receiving a course of omalizumab treatment. There was a maintenance or reduction in the CDR3 spectra of IgE transcripts relative to IgG transcripts of two patients that paralleled clinical improvements, although there was no change in serum IgE levels and no placebo group for this part of the study [45].

Immunogenetic characteristics of immunoglobulin E in non-atopic controls

Full assessment of the provocation of IgE responses in subjects with allergic diseases requires a knowledge of the immunogenetic characteristics of IgE from non-atopic individuals. One hypothesis would be that the immunogenetic characteristics of IgE from non-atopic individuals would reflect a VH, D and JH gene repertoire normal for naïve peripheral B cells with low levels of SHM consistent with little or no germinal centre activity, and no antigenic selection. IgE transcripts from allergic donors have hitherto been compared with published Ig variable domain sequences, or the expressed IgM, IgG or IgA repertoire of the same individual. An early study reported on an analysis of CDR3/FW4 regions of VH-CE transcripts from peripheral B cells of non-atopic individuals obtained by nested PCR techniques [53]. Given the scarcity of IgE-producing B cells in atopic individuals, and more so in controls, others have not expectedly succeeded in amplifying IgE transcripts from non-atopic subjects [31, 35, 40, 43, 46]. Recently, Takhar et al. [54] could amplify IgE transcripts from bronchial biopsies in only two of 10 non-asthmatic atopic controls and one of 10 non-atopic controls compared amplification of IgE from eight of 10 atopic asthmatics and eight of 10 non-atopic asthmatics, but the IgE transcripts from the non-atopic subjects were not analysed. In the study of Efremov et al. [53], there were only four different CDR3 among 32 clones from one of the two non-atopic donors from whom the partial IgE sequences could be amplified. Notably, these authors were unable to obtain IgE amplification products for a further five non-atopic donors. The DH3.03 and JH4.02 that were represented by 33% and 35% of clones, respectively, are frequently observed in the normal Ig repertoire of PBMC [55, 56]. Analysis of full-length variable domain transcripts, rather than just CDR3 regions, would be needed to elucidate patterns of SHM as a measure of germinal centre activity, as well as gene segment usage and clonal relatedness.

We succeeded in amplifying full-length IgE transcripts from PBMC of a non-atopic donor who was non-reactive by skin prick testing (SPT) with a panel of common environmental allergens including animal danders, mite allergens, moulds, and grass and tree pollens (J. M. Davies and R. E. O'Hehir, unpublished observations). There were

Table 2. Gene usage in the VH domain of IgE transcripts from a single non-atopic individual

Clone	Number of repeats	VH	D	JH	CDR3 sequence			VH identity with germline (%)	Length CDR3 (AA)
					VH	D	JH		
1a*	2	3-30.5*01	NF	4*02	CAK	GWYRD	FFDY	97.5-99	12
1b	9	3-30.3*01	NF	4*02	CAK	GYRD	YFDY	96.5-98	12
2a	1	3-23*01	NF	4*02	CAK	MRYDFWSS	YLDY	95	15
2b	1	3-23*01	NF	4*02	CAK	MRYDFWSG	YLDY	97	15
3	1	3-74*01	6-19*01	6*02	CAR	DYGYSSGW	YYYYYGMDV	99.5	20
4	2	3-11*01	1-26*01	6*02	CAR	EQLAKVGGSTTESD	YVVVYGMDV	98	26
5	3	3-21*01	NF	3*02	CAR		FYGGI	98	8
6	2	3-72*01	5-24*01	6*02	CAR	GMGRDGYKSSR	YYYYGMDV	100	22
7	4	4-30.1	NF	6*02	CAR	SCDFLP	LWDV	99	13
8	1	1-3*01	5-24*01	4*02	CAR	WGWFL	YYFDY	93.5	14

*The full-length sequences of these clones have been submitted to GenBank under accession numbers AY846266-AY846275.

NF, matching D gene segment not found in the ImMunoGeneTics database.

VH, variable domain of the heavy chain; CDR, complementarity determining regions; AA, amino acid; D, diversity; JH, joining segment of heavy chain.

26 VH-CE clones sequenced, and only eight showed unique CDR3 sequences, suggesting an *in vivo* IgE repertoire of quite limited clonal diversity (Table 2). This indicates a scarcity of IgE-producing B cells, consistent with the low level of total serum IgE in this donor (21 kU/L). Two pairs of sequences, 1a and 1b and 2a and 2b, had a similar CDR3 and are likely to have arisen by somatic mutations in the CDR3 region. Six of the CDR3 sequences were utilized between two and nine times each, and the patterns of cumulative SHM throughout the VH genes suggest that these were derived from related, but not identical, B cell clones rearranged *in vivo*. The VH genes utilized in the IgE transcripts of this non-atopic donor were those common to the adult circulating IgM repertoire. Most VH-CE clones expressed the major VH gene family 3 and the other major VH gene families, 1 and 4, were also represented once (Table 2). Thirty-six percent and 50% of clones with unique CDR3 utilized the common JH 4.02 and 6.02 genes. The VH genes were on average 98.5% identical to their nearest germline VH gene, reflecting a relatively low level of SHM for class-switched circulating memory B cells (Table 2). Five of eight IgE transcripts from this non-atopic donor did not appear to have undergone significant germinal centre reactions.

The limited clonal diversity of the IgE transcripts of the non-atopic donor (Table 2) can be appreciated when compared with our data on amplified clones from a similar sample of IgM-producing B cells and IgE-producing B cells from patients with seasonal allergy [41]. The level of SHM was less in the variable domains of the IgE transcripts from the non-atopic donor than for IgE transcripts of allergic patients consistent with affinity maturation and allergen exposure in atopic patients (Table 3). Although no difference was observed between the average CDR3 lengths of the IgM and IgE sequences (Table 3), there was a tendency for long CDR3 in a subset of IgE tran-

Table 3. Comparison of IgE transcripts of the non-atopic donor (N) with published¹ IgE and IgM sequences from atopic patients (A, B, C) with seasonal rhinitis

Data set	Unique CDR3/number of sequences (%)	Average identity with germline VH (%)	Average CDR3 length (SD)
IgE of A	12/20 (60)	95.6	17.8 (1.6)
IgE of B	9/19 (47)	90.5	17.6 (4.7)
IgE of C	13/21 (62)	93.5	16.1 (2.7)
IgM of B	24/24 (100)	99.8	16.8 (2.8)
IgE of N	8/26 (31)	98.5	16.3 (6.1)

¹, Davies and O'Hehir [41].

CDR, complementarity determining regions; VH, variable domain of the heavy chain.

scripts from the non-atopic subject in which the JH family 6 gene segment, encoding a long JH segment, was utilized.

Dahlke et al. [25] analysed the frequency and proportion of replacement somatic mutations in IgE transcripts from two non-allergic and one allergic individuals, as well as those listed in IMGT and GenBank. This study provides the largest data set for expressed IgE from allergic and non-allergic donors and includes an analysis of 60 IgE sequences from non-allergic donors. There was a similar frequency of VH gene family usage among IgE and IgG repertoires and, as we observed, there was significantly less SHM in IgE sequences from non-allergic than allergic individuals. The study of Dahlke et al. [25] showed that replacement mutations do not preferentially cluster in CDR for IgE of either non-allergic or allergic donors, implying that antigen selection is not the driving force for accumulation of SHM in IgE sequences, irrespective of whether the IgE transcripts arose from non-atopic or allergic individuals. The provocative question thus arises of whether the cellular interactions and molecular mechanisms responsible for SHM in IgE, irrespective of atopic status, differ from those of IgG involving follicular

dendritic cell presentation of antigen in germinal centres giving rise to antigenic selection.

Limitations of immunogenetic analysis of variable domain of the heavy chain gene usage in atopic individuals

There are some technical limitations to the investigation of the immunogenetics of IgE. These include the number of subjects that can feasibly be analysed in terms of preparation of good quality mRNA, amplification and cloning of the rare IgE VH-CE transcripts, DNA sequencing and immunogenetic analysis. Additionally, there is a limitation imposed by the size of the sample of IgE-producing B cells analysed and whether this is fully representative of the *in vivo* IgE repertoire. It is reasonable to compare the characteristics of IgE-producing B cells from equal volumes of blood of atopic and non-atopic individuals. Also, it is assumed that the array of VH-specific primers used for amplification of VH-CE transcripts is unbiased for preferential amplification of particular Ig genes. During their development, each of these primers was demonstrated to be functional and specific and, when used individually, they did not compete for template during amplification [57]. Using these primers other researchers have successfully amplified a broad range of VH products from different Ig classes [33], and our data showed clonal relatedness among amplified transcripts indicating *in vivo* expansion of related clones rather than amplification bias [41].

In vivo not all VH genes are used stochastically at all stages of development. Certain Ig genes within families are more frequently found in the adult circulating B cell repertoire and databases of rearranged Ig genes: for example, 3-23 and 3-30 for VH family 3, and 4-34 for VH family 4 [58, 59]. Notably, with the exception of the frequently observed increased use of VH1.69 in expressed IgE, the VH genes preferentially utilized in IgE responses of atopic individuals reviewed here are not the common VH genes. JH families 4 and 6 are preferentially utilized, possibly because they favour low-affinity interaction with antigen in unmutated antibodies [52, 55, 56]. This preferential VH gene usage has also been attributed to chromosomal location, proximity to the ribosomal binding site and variation in the flanking recombination signal sequences [59, 60]. Moreover, particular D or J segments yield preferred secondary structures for the CDR3 loop [55, 56].

Analysis of the immunogenetics of IgE is often confounded by sensitization to multiple allergens. Whereas this is frequent, it makes association of VH gene usage with a particular IgE reactivity impractical. The need is for further screening of IgE antibody display libraries to allow gene usage to be linked with antibody specificity [37, 61]. However, with standard techniques of FAb library construction the *in vivo* pairing of heavy and light chains is

not conserved in the library *in vitro*. Even a large and diverse IgE library may not contain the correct heavy and light chain combination that reacts with an allergen of interest [62]. Nonetheless, some studies reviewed here have reported the isolation of allergen-specific antibody fragments [39, 44], but even in these it is not possible to know if the natural heavy and light chain pairing, and reactivity, were preserved.

Location of B cell activation and class switching to immunoglobulin E

Many studies have used peripheral blood as the source of material to examine IgE-producing B cells, yet participation of the mucosal immune system in local and systemic IgE production is important (Table 4). Although the limited availability of human nasal and bronchial mucosal samples can impede investigation of local B cell activation and IgE responses, we emphasize that mucosal IgE production may differ from that of circulating memory B cells emanating from lymphoid germinal centres in several ways. These include the cellular microenvironment, the dominance of IL-4, IL-5 and IL-13, the particle size and form of allergen, and potentially the presence of confounding infectious agents [68-71]. As an example of the difference in IgE responses between mucosa and blood, the bias for VH5 gene usage in IgE responses of 11 patients with AR was significantly greater in the nasal biopsy samples than in PBMC [46].

What are immunoglobulin E-producing B cells doing in allergic disease?

An understanding of both the location and route of development of IgE-producing B cells is important for developing strategies for immunotherapies for allergic diseases. It is accepted that lung dendritic cells take up antigens and then sensitize T cells at local draining lymph nodes rather than at the site of antigen encounter (left of Fig. 1). Dendritic cells loaded with antigen traffic to the spleen or draining lymph node to promote the formation of germinal centres. These centres supply the antigen, T cell and cytokine environment for B cell activation, SHM and CSR to IgE necessary for the development of the adaptive antibody responses. The immunogenetic footprint of the resultant IgE, i.e. clonally related CDR3 with a high level of SHM focused in CDR, would be consistent with antigen-driven B cell responses of the adaptive B-2 B cell repertoire.

New evidence has now established that resident B cells undergo activation, SHM, clonal expansion and CSR to IgE in the local mucosal site rather than IgE-producing B cells being recruited to the lung or nasal mucosa after activation and class switching (Table 4, reviewed in [74] (Fig. 1, top right). B cells dispersed in the mucosal lamina

Table 4. Evidence for mucosal B cell activation, class switching to IgE and SHM

Finding	Patients and tissue	Reference
Dendritic cells and GC occur in the parenchyma of inflamed lung	Murine model of asthma; ova-immunized mice	[63]
Specific IgE, number of IgE-producing B cells and ϵ mRNA levels increased by diesel exhaust and Amb a 1 nasal challenge	Nasal lavage of 13 patients with AR, ragweed allergy	[64]
ϵ I and ϵ C mRNA for IgE detected in mucosal biopsies, suggesting local CSR to IgE	Nasal mucosa of patient with seasonal rhinitis	[65]
Clonally related IgM, IgG and IgE VH mRNA sequences isolated, indicating local CSR and SHM	Bronchial biopsy of patient with asthma	[34]
<i>Ex vivo</i> nasal mucosa exposed to allergen in culture synthesized IL-4 and IL-13 and 30% of B cells expressed ϵ GLT, suggesting that local B cells undergo CSR to IgE	Nasal biopsies from 13 patients with AR and nine controls	[66]
Increase in ϵ I and ϵ C mRNA expression but not B cell numbers in asthmatics vs. controls. Cellular expression of IgE correlated with IL-4 mRNA	Bronchial biopsies; nine atopic asthmatics, nine non-atopic asthmatics, 10 atopic controls and 10 non-atopic controls	[67]
Local CSR to IgE, AID expression indicating SHM, clonal expansion, two patients with related IgE and IgA clones	Nasal biopsies of six patients with AR	[40]
ϵ GLT and ϵ CT transcripts in nasal mucosa, AID expression, isolated B cells and B cells in loosely organized clusters in lamina propria, allergen stimulated CSR to IgE <i>ex vivo</i>	Nasal biopsies in AR, six in season, six out of season	[46]
CSR to IgE and SHM demonstrated by mRNA expression of ϵ GLT, ϵ CT and AID	10 bronchial biopsies each of atopic and non-atopic asthmatics, and atopic and non-atopic controls without asthma	[54]

ϵ I, epsilon promoter exon; ϵ C, epsilon constant region; ϵ GLT, epsilon germline transcript; ϵ CT, epsilon switch circle transcript; AID, activation-induced cytidine deaminase; GC, germinal centre; AR, allergic rhinitis; CSR, class switch recombination; VH, variable domain of the heavy chain; SHM, somatic hypermutation.

propria and germinal centre-like structures have been difficult to detect, although there is evidence of CSR and SHM [67]. Recently, Takhar et al. [46] showed B clusters in the lamina propria of nasal mucosa of patients with AR biopsied during the pollen season. The 'help' for B cell activation is likely to be provided by activated T cells and tissue mast cells expressing CD40L. Nasal biopsy tissue B cells can synthesize IgE, thus providing circumstantial evidence for local IgE synthesis *in vivo* [75]. It is noteworthy that significant IgE synthesis is not readily detected by *in situ* histochemistry despite its presence in the bronchoalveolar lavage (BAL) [76], although KleinJan et al. [77] demonstrated allergen-specific IgE positive B cells and plasma cells in nasal biopsies of patients with AR. Local IgE synthesis is presumed because allergen-specific IgE is detectable in particular sites, notably in secretions of the nose or lung [76]. This points to activated B cells being present at sites of allergic inflammation where they can develop into IgE-producing plasma cells, provide allergen-specific memory B cells to the circulating B cell population and provide IgE to resident mast cells for IgE-facilitated antigen presentation and exacerbation of the allergic cascade [78, 79]. Furthermore, local IgE production may contribute to immunopathogenesis, even in non-atopic patients with either rhinitis or asthma, in the absence of detectable serum IgE or with negative SPTs [80, 81].

The fate of newly and locally matured IgE-producing B cells remains unclear. It is still unknown whether these cells remain in the mucosa and develop into IgE-secreting plasma cells contributing to the local IgE pool, or traffic to the bone marrow and become long-lived plasma cells (Fig. 1, right side). Niederberger et al. [82] showed that local nasal challenge specifically boosts systemic IgE levels by activating memory IgE-producing B cells. This would be consistent with a local activation of B cells that then travel to the bone marrow for IgE production. An increase in allergen-specific IgE in the BAL within 24 h of bronchial allergen challenge indicates local IgE synthesis from resident memory IgE B cells [76]. Earlier research showing that allergen-specific IgE can be restricted to different mucosal sites also indicates that IgE synthesis occurs locally [77]. Finally, IgE-producing plasma cells have been detected in the peripheral blood of atopic individuals, which suggests trafficking of IgE-producing plasma cells to the bone marrow [83].

Do immunoglobulin E-producing B cells follow the traditional B-2 adaptive B cell pathway of development?

In many of the studies reviewed here, IgE responses of allergic individuals have 'immunogenetic footprints' of clonally related CDR3 and SMH in the CDR that is consistent with antigen-driven, affinity-matured and

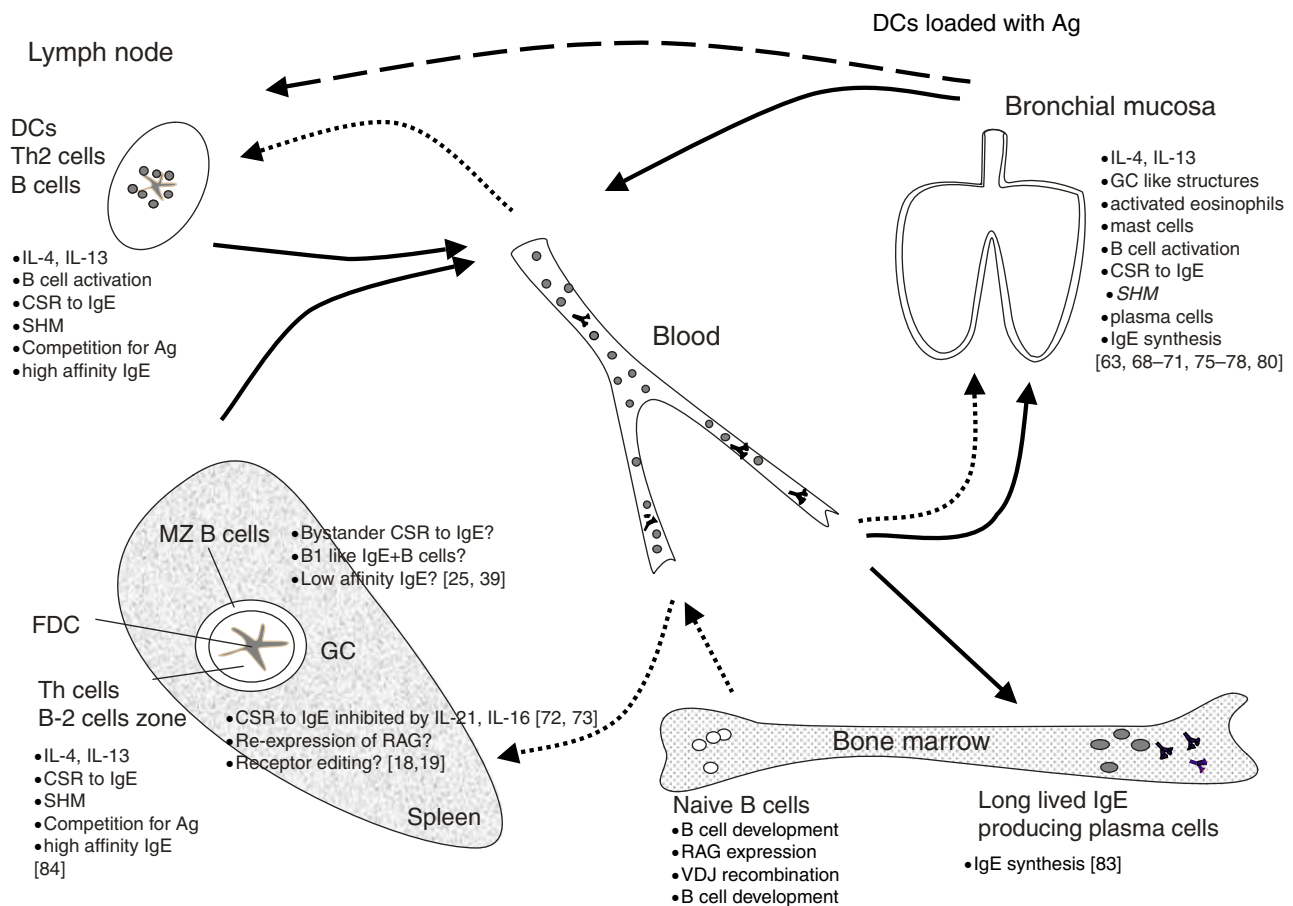


Fig. 1. Routes of activation and development of IgE-producing B cells. Naïve B cells emerge from bone marrow (dotted lines) where they have developed and their Ig genes have rearranged. Naïve circulating B cells encounter antigen presented by activated dendritic cells migrated (dashed lines) to germinal centres of the spleen or draining lymph nodes (top left). With T cell help in the presence of IL-4- and IL-13-activated B cells undergo class switch recombination (CSR) to IgE, Ig variable (V) genes somatically mutate and B cells with antibody of highest affinity for antigen successfully compete for antigen and mature into IgE-producing B cells that enter the lymphatics (solid lines). Alternatively (bottom left), marginal zone B cells, or B1-B cells, in the presence of T-helper 2 cytokines, may switch to IgE and, without antigen competition, give rise to low-affinity B-1-like IgE-producing B cells. In addition, local inflamed mucosa (top right) provides a microenvironment similar to that of germinal centres resulting in antigen presentation, CD40L and cytokine help for local B cell activation, clonal expansion, somatic hypermutation (SHM) and CSR to IgE. This leads to both local resident and circulating IgE-producing memory B cells, and IgE production by local short-lived plasma cells (top right), as well as systemic IgE production by long-lived plasma cells resident in bone marrow (bottom right). DC, dendritic cells; FDC, follicular DC; RAG, recombination activating genes; Th2, T-helper type 2; Ag, antigen; GC, germinal centre [72, 73].

oligoclonally expanded IgE-producing B cell responses (Table 1). All of these studies indicate development of B cells via a traditional B-2-like B cell pathway (top left of Fig. 1). In contrast, data from two studies were more suggestive of an increased stimulation of B cells with immunogenetic features resembling B-1 B cells [39, 41]. The most comprehensive analysis of VH-CE transcripts inferred a lack of antigenic selection in the pattern of SHM of the variable regions of IgE sequences from non-atopic and allergic donors [25], as illustrated in Fig. 1 (bottom left), whereby activation of B1-type or marginal zone B cells might also lead to CSR to IgE in the absence of active antigenic competition, and thus could result in IgE-producing B cells with SHM dispersed throughout the VH

gene rather than clustered in the CDR. These disparate views warrant further investigation, because there remains uncertainty as to whether IgE-producing B cells follow the usual path of the adaptive antibody repertoire. The IgE transcripts analysed by Dahlke et al. [25] included those arising from PBMC and biopsies, but without allowance for differences in B cells arising from these different sources. Also, the VH5 IgE transcripts harbouring FWR mutations would have been overrepresented due to the large number of studies focusing on these transcripts. Clear differences were evident when the positions of SHM in IgE transcripts encoded by VH5 were compared with those of non-VH5 IgE transcripts from nasal biopsies of patients with AR [46]. Mutations were distributed

throughout the entire VH domain in the VH5 transcripts vs. clustering at or near the CDR of non-VH5 transcripts, indicating that different forces may be driving the B cells expressing the VH5 IgE antibodies.

In mice immunized with hapten, specific IgE production occurs in the germinal centre of draining lymph nodes with very few IgE-positive areas in T cell zones, which could be consistent with the accepted model for the development of B cells in the B-2 adaptive antibody repertoire [84]. Erazo et al. [85] present a model of sequential switching from IgG1, the murine equivalent of human IgG4, to IgE with SHM and affinity maturation occurring while the B cell is expressing IgG1 and while the B cell is in the germinal centre. The initiation of class switching to IgE occurs in the germinal centre but IgE-switched B cells rapidly egress from the germinal centre and differentiate to plasma cells [85]. This suggests that IgE B cells are derived from affinity-matured B-2-like B cells but that germinal centre activities of SHM and affinity maturation do not occur in IgE-switched B cells. However, in a bronchial biopsy study of an asthmatic patient Snow et al. [34] identified related IgG4 and IgE clones as well as subclones distinct to each isotype, indicating sequential development of IgG4 to IgE in addition to subsequent SHM of terminally switched IgE-producing B cells. Coker et al. [40] report related IgE and IgA clones from nasal biopsies of a patient with AR, which indicates that SHM occurred before terminal class switching to IgA or IgE. The question of whether B cells are activated and switching to IgE in a germinal centre or germinal centre-like mucosal environment, where SHM and affinity maturation occur under the selective pressure of antigen competition, is an important issue because the extent and site of replacement mutations in the Ig genes and, potentially, the affinity of antibody for cognate antigen would be affected.

Conclusions and future directions

We here look in depth at the immunogenetic aspects of B cells in allergic diseases, among which is asthma that is widely accepted as T cell mediated. B cells are often neglected as a contributor to the underlying immunopathogenesis, yet B cells are highly efficient and potent as antigen presenting cells for the activation of T cells and, in the mucosa, may well contribute to the perpetuation of chronic inflammatory allergic disorders. Local B cells can themselves present antigen and also provide IgE for IgE-facilitated presentation of captured antigen by mast cells and activated eosinophils. Hence, the IgE-producing B cell is important across the spectrum of allergic disorders. Moreover, the efficacy of anti-IgE therapy for the treatment of allergic disease has redirected the spotlight to the B cell, despite a lack of understanding of the precise mechanisms of action of anti-IgE therapy [86]. Blocking

IgG antibodies are also receiving attention as contributors to the regulation of allergic disease [87, 88]. Ascertaining the immunogenetic relationship between IgE and blocking IgG should prove informative of the specificity and development of effective blocking antibodies. Additionally, the possible requirement for IgE-reactive antigen for mucosal immunotherapy vaccination by the sublingual route is now prompting researchers in allergy to give more attention to the processes driving both allergen-specific IgE and IgG responses. Investigation of the immunogenetic characteristics of IgE from patients with allergic disease has already provided interesting new insights into the origin and activation of B cell production. In the near future we are likely to see a revitalized interest in the contribution of IgE antibodies and IgE-producing B cells to the immunopathogenesis of allergic diseases.

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