



Germinal-centre reactions in allergic inflammation

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Primary and secondary immune responses in the germinal centres of lymphoid organs have been studied in the past. There is now compelling evidence of a third stage in the immune response, in 'tertiary lymphoid organs' that develop at sites of chronic inflammation in response to persistent local antigen challenge. Germinal-centre-like reactions are well-documented in the target organs of autoimmune diseases. Here, we review recent evidence that they also occur at sites of allergic inflammation.

IgE and allergic inflammation

Immediate hypersensitivity, the hallmark of the allergic response, is the result of sensitization of mast cells and basophils in mucosal tissues by IgE antibodies [1]. Cross-linking of high-affinity IgE receptor (FcεRI)-bound IgE by allergens activates these cells to release mediators of immediate hypersensitivity and results in more prolonged allergic inflammation. Repeated allergen provocation of affected tissues can result in chronic inflammation, irreversible tissue damage, persistent symptoms and hypersensitivity to further allergen exposure. FcεRI is also expressed by dendritic cells in mucosal tissues. When bound to allergen-specific IgE, it activates these cells in the presence of allergen and facilitates allergen capture for presentation to T-helper (Th) cells.

Conventionally, allergen-specific IgE synthesis is initiated by the interaction between dendritic cells and allergen-specific Th cells in local lymph nodes. These Th cells, in turn, interact with cognate B cells, which divide and form germinal centres. B-cell division is associated with antibody affinity maturation, clonal expansion, class switching and differentiation into memory B cells and antibody-secreting plasma cells. After emerging from lymphoid tissue, some of these plasma cells find a survival niche in the bone marrow [2]. They continue secreting antibodies that prime subsequent immune responses in the germinal centres [3] and also exert effector functions in the periphery.

Compelling evidence, summarized in the next four sections, suggests that, at least for IgE synthesis, this

scenario is geographically less complex, and that all of the immunological events culminating in the production of allergen-specific IgE can occur locally in the inflamed mucosa. A similar scenario is already widely recognized to exist in the target organs of autoimmune disease [4].

Location of IgE synthesis and secretion

What is the origin of IgE antibodies required to sensitize mast cells, basophils and dendritic cells at mucosal surfaces? Is IgE imported from the circulation (Figure 1a, left-hand side), or produced locally in the mucosa, (Figure 1a, right-hand side)?

First, a dilemma: mast cells express FcεRI only after they home to mucosal surfaces [5]; IgE is required for this [1]. So, which comes first, the 'chicken' (IgE synthesis) or the 'egg' (FcεRI expression)? The same dilemma might apply to mucosal dendritic cells. Local IgE synthesis by B cells and plasma cells, abundant at mucosal surfaces [6], could provide a solution to this dilemma.

IgE synthesis and secretion by B cells in the respiratory mucosa was first proposed on the basis of IgE measurements in secretions from patients with allergic rhinitis and asthma (reviewed in [7,8]). Steady-state measurements of IgE were complemented by kinetic studies showing that IgE concentrations increased in nasal secretions more rapidly than in serum in allergic children following re-exposure to allergens after a period of allergen avoidance [9]. The data imply that IgE synthesized and secreted by B cells in the respiratory mucosa spills over into the circulation, rather than the reverse, as is generally assumed. Furthermore, some patients with rhinitis and asthma, who apparently lack circulating, allergen-specific IgE, as detected by conventional skin-prick tests (SPTs) and radio-allergosorbent test (RAST) (Box 1), might, nevertheless, produce allergen-specific IgE in local secretions following local allergen challenge [6,7,10].

Kleinjan *et al.* provided the first direct evidence for this local IgE synthesis [6]. Using dual immunohistochemistry, they were able to detect IgE⁺CD20⁺ B cells and IgE⁺CD138⁺ plasma cells in nasal biopsies from patients with allergic rhinitis. Approximately 4% of the B cells and 12–19% of the plasma cells expressed IgE in the patients with rhinitis, compared with 1% of the B cells and none of the plasma cells in a group of normal subjects. However,

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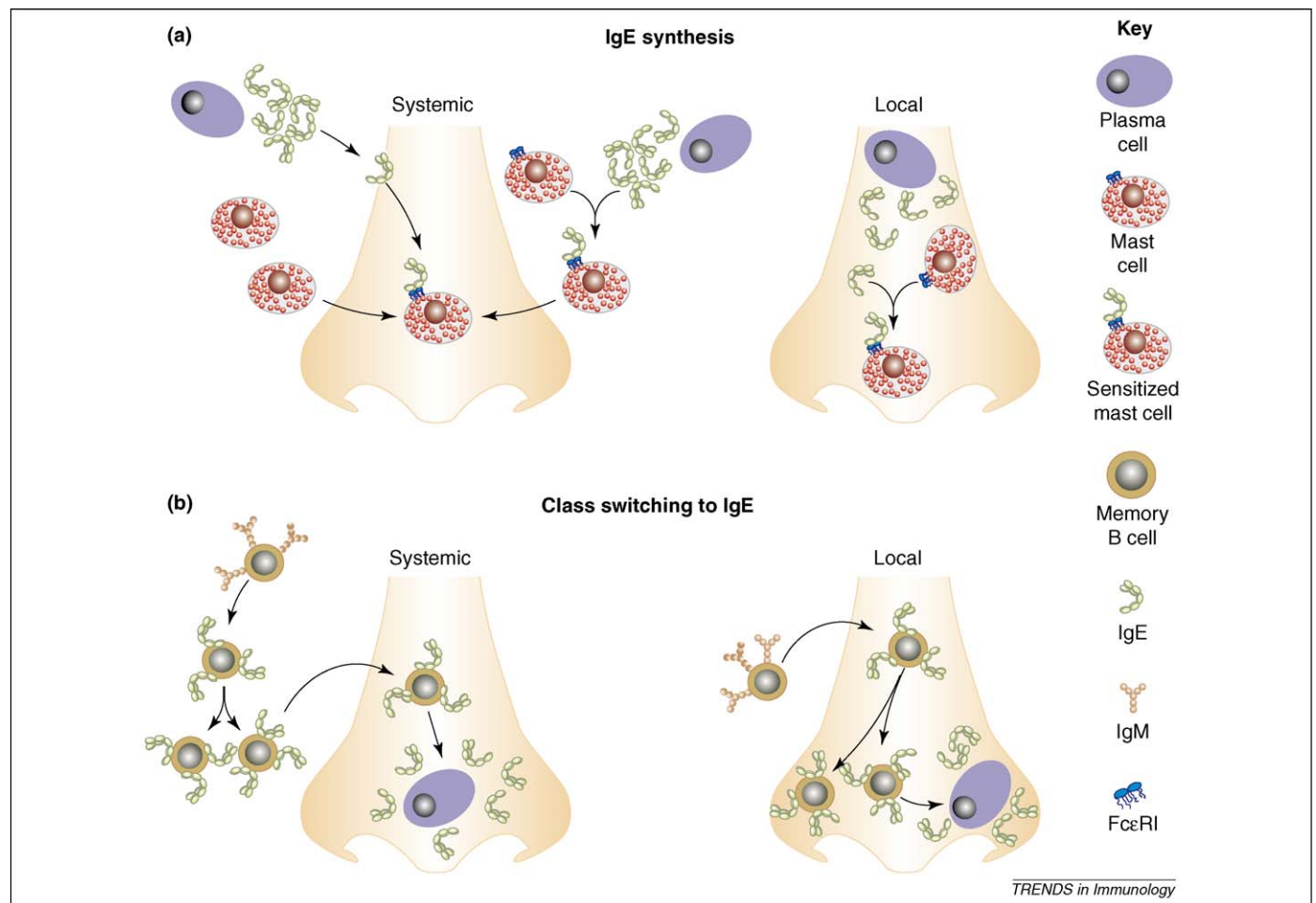


Figure 1. Source of IgE and IgE-expressing plasma cells in the nasal mucosa in rhinitis. **(a)** IgE could be secreted into the circulation by plasma cells in the bone marrow and diffuse into the nasal mucosa, or be passively transported into the mucosa by FcεRI-bearing cells ('systemic'). Alternatively, IgE might be synthesized locally in the nasal mucosa in rhinitis and sensitize neighbouring mast cells in the tissue ('local'). **(b)** In allergic rhinitis, do IgM (IgG or IgA)-expressing memory B cells class-switch to IgE in lymphoid tissue and then migrate to the nasal mucosa ('systemic'), or do they migrate to the nasal mucosa and then switch to IgE ('local') in allergic rhinitis? Experiments designed to distinguish between these possible scenarios are described in this review.

even 1% of IgE-immunoreactive B cells is extremely high, compared with the 0.01% incidence of these cells in the circulation [11]. Therefore, it is clear that IgE-expressing B cells and plasma cells accumulate in the respiratory mucosa in patients with allergic inflammation and, indeed, even in healthy controls. Kleinjan and colleagues also observed that similar proportions of dual-positive IgE⁺CD138⁺ and allergen⁺CD138⁺ plasma cells bound specifically to allergen, suggesting that the majority of IgE⁺ plasma cells in the mucosa of patients with allergic inflammation are specific for allergens [6].

Do these IgE⁺ plasma cells secrete IgE protein? To answer this question, Smurthwaite *et al.* cultured nasal biopsies from patients with seasonal allergic rhinitis (hay fever) sensitized to grass pollen (GP) *ex vivo*, and detected *de novo* IgE synthesis by ELISA and by radioactive amino acid incorporation into IgE [12]. GP-specific IgE synthesis was detectable in biopsies from GP-allergic subjects both in and out of the GP season, suggesting the presence of long-lived plasma cells in the tissue [12]. Thus, the respiratory mucosa might represent a survival niche for IgE-secreting plasma cells [2], or a site of continual replenishment from a source of either local or (more conventionally) distal progenitors [13].

IgE has a short half-life and its persistence requires ongoing synthesis. What contribution do mucosal plasma cells make to this synthesis? When Smurthwaite and colleagues determined the allergen-specific fraction of the total IgE that was synthesized *ex vivo* from nasal biopsies of GP- and house-dust-mite-allergic patients, this was invariably higher than that measured in the serum from the same patients (20–50% versus <10%), and in one case was detectable in the absence of allergen-specific serum IgE [12]. Allergen-specific IgG could not be detected in any of the biopsy supernatants, suggesting extensive switching of mucosal allergen-specific B cells to IgE (and/or possibly IgA) synthesis and/or considerable expansion of IgE-switched cells locally in the tissue.

A significant contribution of local IgE synthesis to the total IgE required to maintain allergic sensitization is also suggested by the rate of IgE synthesis in the nasal mucosa of allergic rhinitis patients [12], which exceeds by more than two orders of magnitude that required to keep local mucosal mast cells permanently sensitized [1]. Overall, the data are compatible with a scenario in which local allergen-specific IgE synthesis sensitizes local mast cells, with the excess 'spilling over' into the circulation at lower concentrations [7,12].

Box 1. Skin-prick test and radioallergosorbent test

Skin-prick test (SPT) and radioallergosorbent test (RAST) are the two most popular and reliable tests to identify allergen-specific IgE in atopic individuals. SPT involves placing a drop of allergen extract on a marked area on the skin (usually the forearm, upper arm or the back). The drop on the skin is then pricked with a lancet. A positive reaction to the SPT occurs when the skin around the needle prick becomes itchy and red with the development of a small blister, called a wheal. The wheal reaches its maximum size in ~15–20 min, and the reaction fades within a few hours. It is caused by histamine release from mast cells sensitized with allergen-specific IgE in the skin. A positive control (histamine) and a negative control (saline) are included in every test. Reaction to an allergen is assessed by the size of the wheal. Up to 25 allergens can be tested simultaneously. Skin tests are usually well tolerated, producing immediate, sensitive and specific results.

RAST is an allergy test that is done on a sample of blood to quantify the amount of serum IgE directed against specific allergens. RAST methodology was invented and marketed in 1974 by Pharmacia Diagnostics AB, Uppsala, Sweden. RAST involves immobilizing an allergen on a solid-phase support such as a microtitre well. Patient serum is incubated with the solid-phase allergen, allowing specific IgE in the serum to bind to the allergen. The allergen–IgE complex is detected with a labelled anti-IgE antibody. The test can be calibrated with reference sera, and is becoming increasingly sensitive, specific and quantitative.

RAST is commonly performed when a patient has extensive skin disease such as eczema, is on medication (anti-histamines or anti-depressants), which antagonizes the effect of histamine, or if a dangerously severe reaction is suspected. Both tests are highly specific, although a skin test is generally more sensitive, less expensive and produces immediate results.

Site of class-switch recombination

Alternative scenarios for the source of IgE-expressing plasma cells in the nasal mucosa are illustrated in Figure 1b. Do B-cells switch to IgE in lymphoid tissue and then migrate into the mucosa, or do they migrate into the mucosa and then switch to IgE (Figure 1b)?

The mechanism of heavy-chain switching to IgE is also illustrated (Figure 2). CD40 ligand expression and the secretion of interleukin (IL)-4 and/or IL-13 by antigen-activated cognate Th2 cells together stimulate class switching to IgE. Dual immunohistochemistry–*in situ* hybridization on respiratory mucosal biopsies from patients with allergic rhinitis [14] and asthma [15,16] demonstrated elevated expression of IL-4 and IL-13 in the tissues, creating a suitable environment in the mucosa for class switching to IgE *in situ*.

To investigate this further, dual immunohistochemistry–*in situ* hybridization was first used to look for ϵ germline gene transcripts (ϵ GLTs) and ϵ -chain mRNA in mucosal B cells. Local allergen provocation of patients with allergic rhinitis resulted in the appearance of ϵ GLTs and ϵ -chain mRNA expression 24 h later [14]. ϵ GLT expression correlated with that of IL-4 mRNA [17]. ϵ GLT and ϵ -chain mRNA were also observed in the bronchial mucosa of asthmatics, interestingly regardless of whether or not they had detectable, allergen-specific IgE in the circulation [18]. GLT expression demonstrates the potential of the B cells to undergo class switching, whereas ϵ -chain mRNA demonstrates that switching has indeed taken place, but neither of these markers provides definitive evidence that class-switch recombination (CSR) has actually occurred locally within the mucosa.

The enzyme activation-induced cytidine deaminase (AID) catalyzes the initial step of CSR, in addition to somatic hypermutation (SHM) in the variable regions of immunoglobulin heavy- and light-chain (V_H and V_L , respectively) genes [19]. Normally, AID is expressed only in germinal-centre B cells [20]. Other enzymes involved in CSR seem to be constitutively expressed in non-lymphoid tissue cells because transfecting the gene encoding AID into fibroblasts induces recombination in these non-lymphoid cells [21]. Thus, like ϵ GLT, AID expression constitutes an additional marker for the potential of B cells to undergo local class switching in tissues.

Switch circles (SCs) are the by-products of CSR (Figure 2). A single SC is generated from genomic DNA following each switch recombination event in a B cell. Because the circle DNA is not replicated at cell division, it is diluted out in the proliferating B-cell population. However, SC DNA is transiently transcribed in multiple copies, facilitating its detection in the B-cell population [22]. Transcription is initiated from the deleted I-exon promoter, now linked to the previously expressed C_H gene. Thus, either SCs or the cDNA sequences complementary to the circle transcripts (CTs) can be used as markers of ongoing (or recent) CSR in the B cells, the particular sequences allowing identification of the switch events that have occurred (Figure 2).

CTs revealing class switching from IgM, IgG1, IgG3 and IgG4 to IgE were observed in nasal biopsies from GP-sensitive patients with hay fever during, but not out, of the pollen season [23]. They were absent from biopsies taken from normal controls [23]. These data suggest that allergen drives class switching to IgE in the respiratory mucosa in allergic rhinitis. To exclude the possibility that B cells switched elsewhere and migrated to the nasal mucosa with their CTs (Figure 1b, left) and to confirm the local stimulation of class switching to IgE by allergen (Figure 1b, right), Takhar and co-workers incubated bisected nasal biopsies taken out-of-season from GP-sensitive patients *ex vivo* with allergen or control medium for 24 h. ϵ GLTs and ϵ CTs appeared predominantly in the samples incubated with allergen, signifying *in situ* B-cell class switching from IgM and IgG to IgE [23]. DNA SCs were also detected in nasal biopsies from ragweed-sensitive patients 24 h after ragweed challenge *ex vivo*, demonstrating local B-cell class switching from IgM and IgG to IgE independently using different techniques and a different allergen [24].

Consistent with these observations, AID mRNA was also detectable in the nasal mucosa of patients with seasonal allergic rhinitis but not normal controls [23]. However, unlike ϵ GLT and ϵ CT, AID mRNA was detectable irrespective of current GP exposure [23]. This might reflect a fundamental abnormality in the nasal mucosa in allergic rhinitis. The results reviewed in this section demonstrate, unequivocally, that switching to IgE can occur in the respiratory mucosa in the absence of cellular traffic from remote sites.

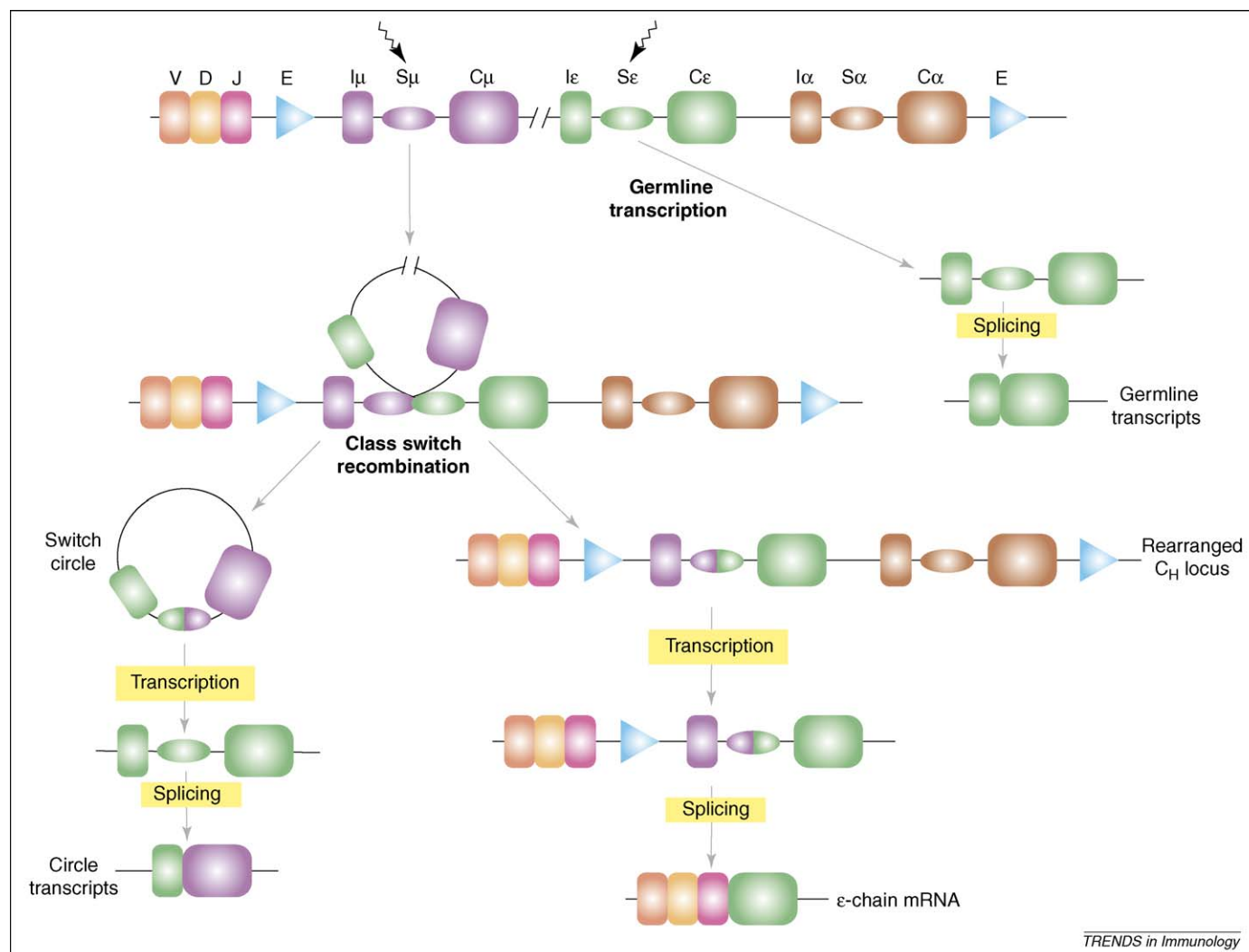


Figure 2. Mechanism of class-switch recombination to IgE, and IgE synthesis. Immunoglobulin heavy-chain constant-region genes (here C μ , C γ , C ϵ and C α), which determine antibody class, are arranged in tandem in the germline configuration downstream of the expressed variable regions (V H or VDJ) and upstream of the 3'-enhancer (E) in the human Ig heavy-chain locus on chromosome 14. Each heavy-chain germline gene comprises an intervening (I) exon (narrow rectangles) with its I H promoter, a GC-rich tandem repeat, called a switch (S) region (ovals), containing potential DNA breakpoints, and the constant-region exons (broad rectangles). IL-4 and IL-13 stimulate ϵ germline gene transcription. Transcription of the ϵ germline gene leads, through splicing, to ϵ germline gene transcripts (GLTs). GLTs contain stop codons in all three reading frames, precluding translation into protein; however, the GLTs are needed for CSR. CSR occurs by deletional rearrangement with break points in the two participating S regions (zig-zag arrows). During recombination, for example from IgM to IgE, the broken ends of S μ and S ϵ join to form a hybrid S junction to express ϵ -chain mRNA and protein. A by-product of this reaction is the looped-out and deleted intervening sequence, the ends of which are joined to form a reciprocal hybrid S junction. The extrachromosomal circular DNA molecule, known as a switch circle (SC), is not replicated during cell division, and can be lost following B-cell proliferation. SCs contain the I ϵ promoter upstream of the targeted S ϵ region, the S ϵ -S μ hybrid junction and C μ , allowing the expression of multiple switch circle transcripts (CTs). Owing to the rapid decay of CTs in the B-cell population, they are convenient markers for ongoing or recent CSR from IgM to IgE. The presence of CTs is an irrefutable sign of the irreversible process of CSR.

Local somatic hypermutation and clonal expansion of IgE-expressing B cells

SHM results from the stepwise accumulation of single-nucleotide substitutions in the V-region of immunoglobulin genes, generally in the complementarity-determining regions (CDRs) (Box 2). This enables the genealogy of a B cell to be traced, relying on the unique clonal signature generated by V(D)J recombination in the progenitor cell. These mutations generate the substrates for the affinity maturation and clonal expansion of antigen-specific B cells before their differentiation into memory B cells and antibody-secreting plasma cells in germinal centres [25]. The mechanism of SHM entails DNA cleavage and re-ligation and, as in CSR, the activity of AID [19].

The first evidence for local SHM in allergic disease was reported in relation to asthma. Snow *et al.* noted the

presence of V ϵ sequences containing multiple nucleotide substitutions, organized into clonal families, in a bronchial biopsy taken from an asthma patient [26]. However, the same authors observed similar V ϵ clonal families in the peripheral blood [27,28] and splenic [29] B cells of asthmatics, detracting from the possibility of local clonal expansion of B cells.

Definitive results were obtained in a study of nasal biopsies from six patients with allergic rhinitis [30]. All of the biopsies contained highly mutated V ϵ sequences in multiple copies. Two out of six of these biopsies also contained three IgE⁺ B-cell clonal families [30]. One biopsy featured a V ϵ 3 and a V ϵ 5 clonal family. The second biopsy also contained a V ϵ 5 clonal family, plus related IgA2 clones, providing further evidence of local CSR. No IgE sequences were seen in the nasal mucosa of a non-allergic

Box 2. Somatic hypermutation

Somatic hypermutation (SHM) is the incorporation of point mutations within the immunoglobulin V-region genes, which serve as the basis for affinity maturation and effective immune responses. The process is initiated by antigen stimulation, occurs in the dark zone of germinal centres, and requires functional AID [49]. AID preferentially deaminates cytidine within the sequence motif WRC, which explains the tendency for 'hotspots' of SHM to occur at both WRC and its reverse complement GYW in the V gene [50]. The higher concentration of these motifs within the CDRs compared with the framework regions (FWRs) of germline V segments contributes to the greater mutability of the CDRs and suggests that intrinsic SHM evolved (i) towards increasing variability in antigen binding sites, and (ii) to avoid disrupting antibody structure by the mutation of framework residues.

Within the germinal centres of classical lymphoid tissues, B cells expressing hypermutated antibodies are subjected to selection through antigen presentation by follicular dendritic cells, where cells with decreased affinity, due to unfavourable mutation, undergo apoptosis. Those with improved antigen affinity might differentiate into memory B cells or plasma cells, or return to the germinal centre to undergo further rounds of cell division and SHM, resulting in clonal B-cell families with similar and diverse V-gene mutations, as observed in the nasal mucosa [30].

individual who had high levels of circulating IgE [30], excluding the possibility that IgE-expressing B cells automatically home to the nasal mucosa.

The accumulation of clonal B-cell families at mucosal surfaces supports the conclusion that somatic mutation occurs in the periphery because it is highly unlikely that B cells undergoing multiple rounds of somatic mutation and clonal expansion in lymphoid tissues will produce progeny which migrate back to one small region of tissue, as would be contained in a biopsy. This argument was substantiated by the examination of two adjacent biopsies from a patient with allergic rhinitis [31]. One biopsy contained 11/11 unrelated V ϵ 5 sequences, whereas the adjacent biopsy contained 0/4 V ϵ 5 sequences [31]. V α 5 sequences were also enriched in the same biopsy containing the V ϵ 5 sequences, but absent in the adjacent biopsy [31]. None of the V α sequences were related to the others, or to any of the V ϵ sequences. This geographical isolation of B-cell clones expressing the same V_H germline gene family in the nasal mucosa is reminiscent of the oligoclonal B-cell clusters in germinal centres microdissected from lymphoid tissue [32,33].

The observations of two out of three V ϵ 5 clonal families in the survey of six allergic rhinitis patients [30], and the clustering of V_H5 clones [31], are of interest. V_H5 is one of the smallest of the seven V_H families, comprising only one or two of the 50 functional germline V_H genes in the human V_H germline gene repertoire, and represented proportionately in normal blood B cells [34]. When the frequency of V ϵ 5 clones (counting each single clone and each clonal family only once) was determined, a tenfold over-representation of V ϵ 5 was observed in the nasal mucosa, compared with the normal blood B-cell repertoire. There was no significant elevation of V ϵ 5 expression in the blood B cells of the six allergic rhinitis patients, underlining the local nature of the bias in the target organ [31]. A similar bias towards the mucosal expression of V ϵ 5 was also found in asthma [26,27,29] and atopic dermatitis [35].

B-cell superantigens have been associated with the selection of different V_H families, leading to massive B-cell proliferation, regardless of antigen specificity [36]. To date, B-cell superantigens binding to members of the V_H3, V_H4, V_H6 and V_K families have been discovered [36]. *Staphylococcus aureus*, a notorious source of enterotoxins (SAEs) that function as superantigens, permanently colonizes the nasal mucosa of a fifth of the population [37]. *S. aureus* enterotoxin A (SEA) and D (SED) select for V_H3 and V_H4, respectively. SAEs have been identified as a causal factor in atopic dermatitis [38,39] and allergic rhinitis [40]. IgE antibodies against *S. aureus* enterotoxin B (SEB) in serum have been correlated with the severity of asthma [41]. Thus, superantigens might be one stimulus for B-cell clonal expansion at the mucosal surfaces of the skin and respiratory tract. Cell proliferation could, in turn, account for local CSR, ultimately to IgE [42].

Local formation of germinal-centre-like structures in allergic inflammation

The evidence presented in this review supports the possibility of germinal-centre-like reactions in allergic disease. Germinal-centre-like structures develop slowly during the progression of autoimmune disease [4]. However, even the most highly developed structures lack the typical encapsulation of cell clusters and channels for the orderly trafficking of cells and molecules seen in the classical germinal-centre reactions in lymphoid tissue [4]. The absence of these features in autoimmune disease might be of functional significance: the cells and molecules involved in local germinal-centre reactions are probably all available in one place. Corraling and traffic control in these areas should be superfluous and could actually be inhibitory [4]. In fact, there are preliminary indications that similar structures might develop in the nasal mucosa in rhinitis [23] and nasal polyposis [43].

Beyond the respiratory tract

Extrapolating from autoimmune disease, Aloisi and Pujol-Borrell have predicted that germinal-centre reactions might be a general phenomenon associated with chronic inflammation [4]. The data described here relating to allergic inflammation in the respiratory tract and skin are analogous to those cited for germinal-centre reactions in autoimmune disease, and provide support for this generalization. In earlier work using a mouse model of asthma, Chvatchko *et al.* demonstrated the development of germinal centres in the lung following sensitization, then multiple intra-tracheal challenge of mice with ovalbumin [44]. They observed the development of pathophysiological changes characteristic of asthma and airway hyper-responsiveness. Autoimmune disease is associated with Th1, and allergic disease with Th2 responses, involving class switching to IgG in Th1 and IgE in Th2. Nevertheless, local class switching and antibody production are not restricted to these two isotypes, because class switching to IgA occurs in the murine gastrointestinal tract, a location under constant antigenic challenge [45]. Furthermore, ϵ GLT and IL-4 mRNA expression has been detected in the human intestinal mucosa, to a greater degree in patients with food allergy [46]. Skin, the target organ of atopic

dermatitis, with a combined Th1 and Th2 aetiology and characterized by enhanced IgE antibody production directed against aero-allergens and food allergens, is uncharted territory.

Little is known about the mechanisms that initiate and maintain germinal-centre reactions in the target organs of autoimmune and allergic diseases. It should be interesting to explore the similarities and differences between these mechanisms in the two types of disease. It might be particularly illuminating to compare and contrast the control mechanisms influencing immunoglobulin switching in the respiratory and gastrointestinal tracts, because oral tolerance is a well-recognized phenomenon typified by the recent therapeutic success of sublingual allergen immunotherapy [47,48]. A scenario whereby mucosal IgE production contributes significantly to total IgE production underlines the value of topical treatment, for example, with corticosteroids, for allergic diseases. With novel therapies, such as anti-IgE antibody, it is pertinent to recognize that local IgE production might not always be reflected in circulating allergen-specific IgE concentrations.

Concluding remarks

We have reviewed the evidence that B-cell somatic hypermutation, clonal expansion, class switching to IgE and plasma-cell IgE synthesis occur in the nasal mucosa in allergic rhinitis. We have also presented evidence that these sites could be major contributors to ongoing IgE synthesis, particularly after allergen exposure. Interestingly, it would seem that these diseases are marked by the abnormal expression of AID, persistent IgE-antibody synthesis and the preservation of FcεRI expression on, and sensitization of, mast cells and dendritic cells in the tissue. Mechanistic differences between normal immune responses in lymphoid tissues and allergic responses in the periphery have yet to be investigated. It might be possible to design topical inhibitors that select for processes that occur in the target organs.

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References

- Gould, H. *et al.* (2003) The biology of IgE and the basis of allergic disease. *Annu. Rev. Immunol.* 21, 579–628
- Moser, K. *et al.* (2006) Stromal niches, plasma cell differentiation and survival. *Curr. Opin. Immunol.* 18, 265–270
- Tew, J.G. *et al.* (2001) Follicular dendritic cells: beyond the necessity of T-cell help. *Trends Immunol.* 22, 361–367
- Aloisi, F. and Pujol-Borrell, R. (2006) Lymphoid neogenesis in chronic inflammatory diseases. *Nat. Rev. Immunol.* 6, 205–217
- Prussin, C. and Metcalfe, D.D. (2006) IgE, mast cells, basophils, and eosinophils. *J. Allergy Clin. Immunol.* 117, S450–S456
- Kleinjan, A. *et al.* (2000) Local production and detection of (specific) IgE in nasal B-cells and plasma cells of allergic rhinitis patients. *Eur. Respir. J.* 15, 491–497
- Smurthwaite, L. and Durham, S.R. (2002) Local IgE synthesis in allergic rhinitis and asthma. *Curr. Allergy Asthma Rep.* 2, 231–238
- Durham, S.R. *et al.* (1997) IgE regulation in tissue. In *IgE Regulation Molecular Mechanisms* (Vercelli, D., ed.), pp. 21–36, Wiley
- Sensi, L.G. *et al.* (1994) Changes in nasal specific IgE to mites after periods of allergen exposure-avoidance: a comparison with serum levels. *Clin. Exp. Allergy* 24, 377–382
- Powe, D.G. *et al.* (2003) 'Entropy': localized mucosal allergic disease in the absence of systemic responses for atopy. *Clin. Exp. Allergy* 33, 1374–1379
- King, C.L. *et al.* (1991) Frequency analysis of IgE-secreting B lymphocytes in persons with normal or elevated serum IgE levels. *J. Immunol.* 146, 1478–1483
- Smurthwaite, L. *et al.* (2001) Persistent IgE synthesis in the nasal mucosa of hayfever patients. *Eur. J. Immunol.* 31, 3422–3431
- Hiepe, F. and Radbruch, A. (2006) Is long-term humoral immunity in the mucosa provided by long-lived plasma cells? A question still open. *Eur. J. Immunol.* 36, 1068–1069
- Cameron, L.A. *et al.* (1998) Expression of IL-4, Cε RNA, and Iε RNA in the nasal mucosa of patients with seasonal rhinitis: effect of topical corticosteroids. *J. Allergy Clin. Immunol.* 101, 330–336
- Humbert, M. *et al.* (1996) IL-4 and IL-5 mRNA and protein in bronchial biopsies from patients with atopic and nonatopic asthma: evidence against 'intrinsic' asthma being a distinct immunopathologic entity. *Am. J. Respir. Crit. Care Med.* 154, 1497–1504
- Humbert, M. *et al.* (1997) Elevated expression of messenger ribonucleic acid encoding IL-13 in the bronchial mucosa of atopic and nonatopic subjects with asthma. *J. Allergy Clin. Immunol.* 99, 657–665
- Cameron, L. *et al.* (2000) Local synthesis of ε germline gene transcripts, IL-4, and IL-13 in allergic nasal mucosa after *ex vivo* allergen exposure. *J. Allergy Clin. Immunol.* 106, 46–52
- Ying, S. *et al.* (2001) Local expression of ε germline gene transcripts and RNA for the ε heavy chain of IgE in the bronchial mucosa in atopic and nonatopic asthma. *J. Allergy Clin. Immunol.* 107, 686–692
- Muramatsu, M. *et al.* (2000) Class switch recombination and hypermutation require activation-induced cytidine deaminase (AID), a potential RNA-editing enzyme. *Cell* 102, 553–563
- Muramatsu, M. *et al.* (1999) Specific expression of activation-induced cytidine deaminase (AID), a novel member of the RNA-editing deaminase family in germinal centre B cells. *J. Biol. Chem.* 274, 18470–18476
- Okazaki, I.M. *et al.* (2002) The AID enzyme induces class switch recombination in fibroblasts. *Nature* 416, 340–345
- Kinoshita, K. *et al.* (2001) A hallmark of active class switch recombination: transcripts directed by I promoters on looped-out circular DNAs. *Proc. Natl. Acad. Sci. U. S. A.* 98, 12620–12623
- Takhar, P. *et al.* (2005) Allergen drives class switching to IgE in the nasal mucosa in allergic rhinitis. *J. Immunol.* 174, 5024–5032
- Cameron, L. *et al.* (2003) ScSμ and ScSγ switch circles in human nasal mucosa following *ex vivo* allergen challenge: evidence for direct as well as sequential class switch recombination. *J. Immunol.* 171, 3816–3822
- Tarlinton, D.M. and Smith, K.G.C. (2000) Dissecting affinity maturation: a model explaining selection of antibody-forming cells and memory B cells in the germinal centre. *Immunol. Today* 21, 436–441
- Snow, R.E. *et al.* (1999) Analysis of immunoglobulin E V_H transcripts in a bronchial biopsy of an asthmatic patient confirms bias towards V_H5, and indicates local clonal expansion, somatic mutation and isotype switch events. *Immunology* 98, 646–651
- Snow, R.E. *et al.* (1997) Pattern of usage and somatic hypermutation in the V_H5 gene segments of a patient with asthma: implications for IgE. *Eur. J. Immunol.* 27, 162–170
- Snow, R.E. *et al.* (1998) Clonally related IgE and IgG4 transcripts in blood lymphocytes of patients with asthma reveal differing patterns of somatic mutation. *Eur. J. Immunol.* 28, 3354–3361
- Snow, R.E. *et al.* (1995) Analysis of Ig V_H region genes encoding IgE antibodies in splenic B lymphocytes of a patient with asthma. *J. Immunol.* 154, 5576–5581
- Coker, H.A. *et al.* (2003) Local somatic hypermutation and class switch recombination in the nasal mucosa of allergic rhinitis patients. *J. Immunol.* 171, 5602–5610
- Coker, H.A. *et al.* (2005) Biased use of V_H5 IgE-positive B cells in the nasal mucosa in allergic rhinitis. *J. Allergy Clin. Immunol.* 116, 445–452
- Kuppers, R. *et al.* (1993) Tracing B cell development in human germinal centres by molecular analysis of single cells picked from histological sections. *EMBO J.* 12, 4955–4967
- Jacob, J. *et al.* (1991) Intracloal generation of antibody mutants in germinal centres. *Nature* 354, 389–392
- Brezinschek, H.-P. *et al.* (1995) Analysis of the heavy chain repertoire of human peripheral B-cells using single-cell polymerase chain reaction. *J. Immunol.* 155, 190–202

- 35 van der Stoep, N. *et al.* (1993) Molecular evolution of the human immunoglobulin E response: high incidence of shared mutations and clonal relatedness among ϵ V_H5 transcripts from three unrelated patients with atopic dermatitis. *J. Exp. Med.* 177, 99–107
- 36 Silverman, G.J. and Goodyear, C.S. (2006) Confounding B-cell defences: lessons from a staphylococcal superantigen. *Nat. Rev. Immunol.* 6, 465–475
- 37 Kluytmans, J. *et al.* (1997) Nasal carriage of *Staphylococcus aureus*: epidemiology, underlying mechanisms, and associated risks. *Clin. Microbiol. Rev.* 10, 505–520
- 38 Leung, D.Y. (1995) Atopic dermatitis: the skin as a window into the pathogenesis of chronic allergic diseases. *J. Allergy Clin. Immunol.* 96, 302–318
- 39 Skov, L. *et al.* (2000) Application of *Staphylococcal* enterotoxin B on normal and atopic skin induces up-regulation of T cells by a superantigen-mediated mechanism. *J. Allergy Clin. Immunol.* 105, 820–826
- 40 Riechelmann, H. *et al.* (2005) Nasal carriage of *Staphylococcus aureus* in house dust mite allergic patients and healthy controls. *Allergy* 60, 1418–1423
- 41 Bachert, C. *et al.* (2003) IgE to *Staphylococcus aureus* enterotoxins in serum is related to severity of asthma. *J. Allergy Clin. Immunol.* 111, 1131–1132
- 42 Tangye, S.G. *et al.* (2002) Isotype switching by human B cells is division-associated and regulated by cytokines. *J. Immunol.* 169, 4298–4306
- 43 Gevaert, P. *et al.* (2005) Organization of secondary lymphoid tissue and local IgE formation to *Staphylococcus aureus* enterotoxins in nasal polyp tissue. *Allergy* 60, 71–79
- 44 Chvatchko, Y. *et al.* (1996) Germinal centre formation and local immunoglobulin E (IgE) production in the lung after an airway antigenic challenge. *J. Exp. Med.* 184, 2353–2360
- 45 Fagarsan *et al.* (2001) *In situ* class switching and differentiation to IgA-producing cells in the gut lamina propria. *Nature* 413, 639–643
- 46 Coeffier, M. *et al.* (2005) ϵ germ-line and IL-4 transcripts are expressed in human intestinal mucosa and enhanced in patients with food allergy. *Allergy* 60, 822–827
- 47 Allam, J.-P. *et al.* (2006) Comparative analysis of nasal and oral mucosa dendritic cells. *Allergy* 61, 166–172
- 48 Marcucci, F. *et al.* (2005) Dose dependence of immunological response to sublingual immunotherapy. *Allergy* 60, 952–956
- 49 Martomo, S.A. and Gearhart, P.J. (2006) Somatic hypermutation: subverted DNA repair. *Curr. Opin. Immunol.* 18, 243–248
- 50 Petersen-Mahrt, S. (2005) DNA deamination in immunity. *Immunol. Rev.* 203, 80–97