

THE BIOLOGY OF IgE AND THE BASIS OF ALLERGIC DISEASE

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■ **Abstract** Allergic individuals exposed to minute quantities of allergen experience an immediate response. Immediate hypersensitivity reflects the permanent sensitization of mucosal mast cells by allergen-specific IgE antibodies bound to their high-affinity receptors (FcεRI). A combination of factors contributes to such long-lasting sensitization of the mast cells. They include the homing of mast cells to mucosal tissues, the local synthesis of IgE, the induction of FcεRI expression on mast cells by IgE, the consequent downregulation of FcγR (through an insufficiency of the common γ-chains), and the exceptionally slow dissociation of IgE from FcεRI. To understand the mechanism of the immediate hypersensitivity phenomenon, we need explanations of why IgE antibodies are synthesized in preference to IgG in mucosal tissues and why the IgE is so tenaciously retained on mast cell–surface receptors. There is now compelling evidence that the microenvironment of mucosal tissues of allergic disease favors class switching to IgE; and the exceptionally high affinity of IgE for FcεRI can now be interpreted in terms of the recently determined crystal structures of IgE–FcεRI and IgG–FcγR complexes. The rate of local IgE synthesis can easily compensate for the rate of the antibody dissociation from its receptors on mucosal mast cells. Effective mechanisms ensure that allergic reactions are confined to mucosal tissues, thereby minimizing the risk of systemic anaphylaxis.

OVERVIEW

An allergic reaction is initiated when an antigen crosslinks immunoglobulin E (IgE) antibodies bound to their high-affinity Fc receptor (FcεRI) on tissue mast cells or blood basophils (1). The immediate reaction, taking effect within minutes of allergen provocation, results in the release of mediators that lead to symptoms characteristic of the target organ. A late-phase response associated with the influx of T cells, monocytes, and eosinophils may ensue some hours later. Hayfever,

asthma, reactions to food, and eczema are the most common allergic responses, caused by mast cell activation in mucosal tissues of, respectively, the nose, lung, gut, and skin. Blood basophils are the IgE effector cells in the rare, but more dangerous, manifestation of systemic anaphylaxis.

IgG antibodies mediate hypersensitivity reactions in animal models but have never been found to display this activity in humans. A part, at least, of the explanation for this fundamental divergence lies in the different physical properties of IgE and IgG, the relevant features of which can now be discerned from recently determined high-resolution crystal structures of the Fc, Fc receptor (FcR), and Fc:FcR complexes. These structures afford, in particular, an explanation for the uniquely high affinity of the IgE-Fc ϵ RI complex.

Why are allergic reactions confined to particular target organs? Clearly the homing propensity of mast cells to mucosal tissues (2, 3) is essential. The tenacity of IgE binding to Fc ϵ RI on mast cells is important, but an additional important factor is the persistent local synthesis and secretion of IgE. This synthesis and secretion maintains the sensitization of the mast cells by replacing IgE lost by degradation or by dissociation and diffusion into the circulation or secretions. In addition the mast cell mediators induce changes in the local vasculature that result in the influx and activation of inflammatory cells, effectively targeting the late phase response to sites of allergen provocation.

The microenvironment of mucosal tissues favors the synthesis of IgE at the expense of IgG. IgE regulation of Fc ϵ RI expression on mast cells couples the expression of receptor to that of IgE, and thus local synthesis and secretion of IgE leads to the upregulation of Fc ϵ RI on neighboring mast cells. Competition between Fc ϵ RI and the IgG receptor (Fc γ R) in the mast cell for their common γ -chain may then lead to a concerted downregulation of Fc γ R.

The basis for local IgE synthesis can be inferred in outline. The expression of IgE requires heavy-chain switching from IgM, often by way of IgG, to IgE by somatic recombination of the germline genes in B cells. Class switching is linked to cell division, and the IgE switch requires more cycles than the switch to IgG. Cells may drop out at any stage of this process by terminal differentiation of the B cells into immunoglobulin-secreting plasma cells or by apoptosis. Local conditions in the mucosal tissues of allergic individuals evidently favor class switching to IgE, and the rate at which it is synthesized and secreted can be calculated to overcome the loss of bound antibody from the mast cells.

IgE and IgE receptors (Fc ϵ RI and the low-affinity receptor, Fc ϵ RII/CD23) participate in the afferent phase of the immune response to allergens. Langerhans cells in epidermal tissues internalize and process allergens from the environment and transport peptide-MHC class II antigen complexes to the local lymphoid tissues to amplify the immune response and reimplant memory of the allergen for future responses. Restriction of these activities to local lymphoid and mucosal tissues at sites of exposure to antigen constitutes an essential safeguard against systemic anaphylaxis.

PROTEINS OF THE IgE NETWORK

IgE

There are nine antibody classes (isotypes) in humans: IgM, IgD, IgG1, IgG2, IgG3, IgG4, IgA1, IgA2, and IgE. All have a similar structure consisting of heavy (H) and light (L) chains with variable (V) and constant (C) regions made up of Ig domains, as shown in Figure 1A. H-chains differ in the number of CH domains, with three in IgD, IgG, and IgA, and four in IgM and IgE. Ig domains each contain about 110 amino acids and comprise a β -sheet “sandwich” with three and four β -strands in the C-type topology (4) (Figure 1B). The V regions of the L- and H-chains make up a pair of identical antigen-binding sites. These, together with the adjacent CH domain pair, constitute the Fab region of the antibody. The remaining Ig domains pair off to create the Fc region of the antibody, which contains the FcR binding sites.

IgE is characterized by ϵ H-chains, which contain one variable (V) H-chain (VH) and four constant region (C ϵ 1-4) domains (Figure 1A). IgD, IgG, and IgA have a flexible hinge in place of the CH2 domain in IgM and IgE, but at least one inter-heavy-chain disulfide bond is conserved between CH2 and the hinge. Because of the missing Ig domain, the CH2 and CH3 domains of IgD, IgG, and IgA are homologous, respectively, to CH3 and CH4 in IgM and IgE. The extra domain (C ϵ 2) in IgE is a critical determinant in its distinctive physical properties and isotype-specific functions (see Crystal and NMR Structures, below).

The V regions expressed in a B cell determine its antigen specificity, and those in the whole B cell population determine the individual's antibody repertoire at any given time. The VH repertoire of IgE in allergic individuals, however, differs from that of other antibody classes. This may reflect the action of allergens as superantigens (see Somatic Hypermutation of IgE VH Regions, below).

Mature B cells begin by expressing IgM but may switch to another antibody class or sequentially to two or more other antibody classes upon antigen activation (see H-Chain Class Switching to IgE, below). Antibody classes lend diversity to the effector functions by enabling the antibody to bind to specific FcR, distributed unequally among the various effector cell types, concentrated in different microenvironments. The half-life of IgE in serum is 3 days, compared with 20 days for IgG, but much of the IgE is sequestered in tissues (5). Thus immune surveillance by IgG occurs primarily in the circulation, IgE in tissues, and IgA in secretions.

IgE is the least abundant antibody class in serum, with a concentration of ~ 150 ng/ml, compared with 10 mg/ml for IgG in the circulation of normal (“nonatopic”) individuals. Serum IgE concentrations reflect the number of circulating B cells committed to IgE synthesis (6, 7). In certain parasitic diseases and in the hyper-IgE syndrome, serum IgE concentrations may be up to three orders of magnitude higher than normal without signs of allergic disease (8).

IgE concentrations in the circulation may reach over 10 times the normal level in “atopic” individuals, who have increased risk of developing allergies. Allergen-specific IgE antibody concentrations are generally more closely correlated with

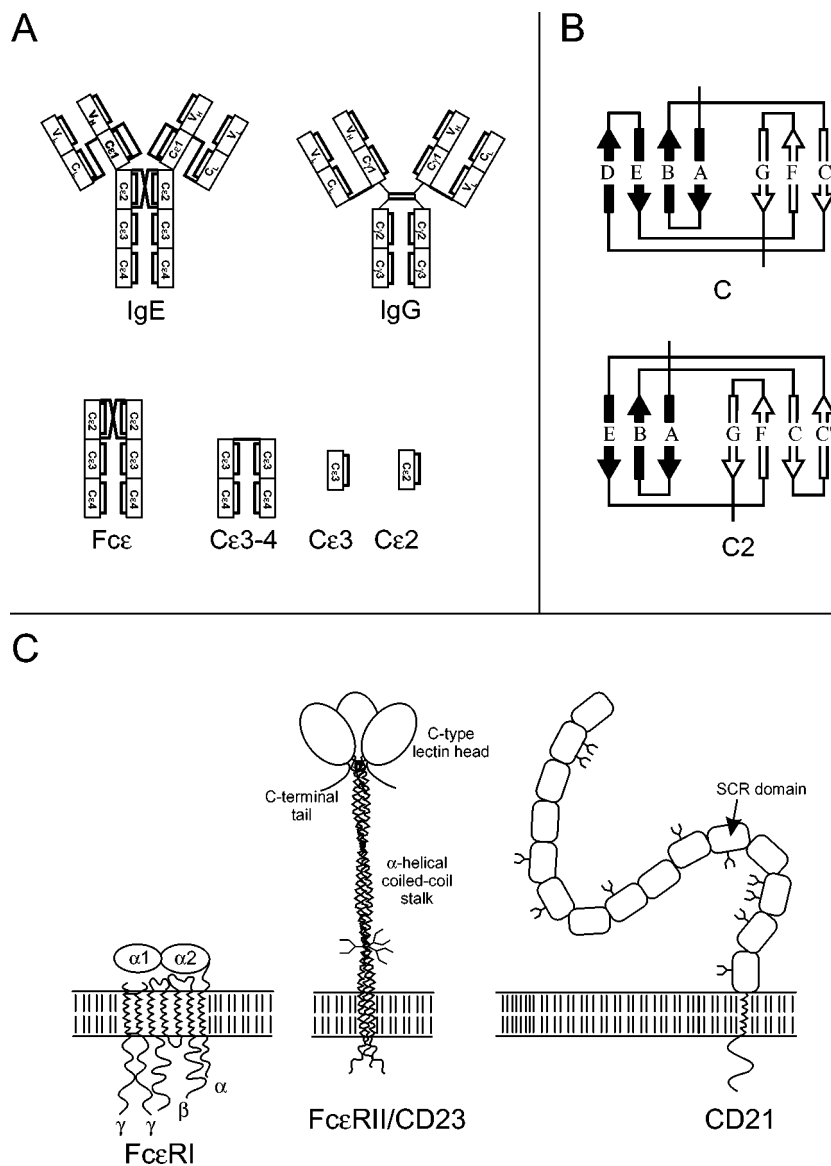


Figure 1 IgE and its receptors. (A) The domain structure of IgE, IgG, and isolated fragments of IgE used in structural studies. (B) The β strand topology of C and C2 type immunoglobulin domains. (C) Schematic representations of the IgE receptors Fc ϵ RI, Fc ϵ RII/CD23, and the CD23 coreceptor CD21.

symptoms and may be over 1000 times higher than the minimum level of detection (~ 0.6 ng/ml) found in the majority of healthy subjects (9). However, in some individuals with hayfever or asthma IgE antibodies are detectable only in secretions from the target organ. This may reflect the occurrence of local IgE antibody synthesis against autoantigens in the target organ (see Local Regulation of IgE, below).

Antibodies are expressed in a developmental stage-dependent manner as membrane-bound (mIg) and secreted (Ig) forms, arising from differential splicing of a common mRNA precursor. The mIg is associated with α - and β -chains, which transduce signals from the antigen (10). Signal transduction by mIgE may play an important role in isotype determination (see Multiple Functions of CD23, and H-Chain Class Switching to IgE, below).

Fc ϵ RI

Fc ϵ RI, the high-affinity IgE receptor, is abundant (200,000 molecules/cell) in mast cell and basophil membranes and is expressed at much lower levels in Langerhans cell, monocyte, platelet, and eosinophil membranes (see Effector Functions of Fc ϵ RI, below). Fc ϵ RI is expressed as an $\alpha\beta\gamma_2$ heterotetramer in mast cells and basophils (and possibly eosinophils), and as an $\alpha\gamma_2$ heterotrimer in monocytes and platelets (Figure 1C).

Fc ϵ RI shares a number of attributes with most other FcRs (11, 12). Like Fc ϵ RI, Fc γ RI, Fc γ RIII, and Fc α R are $\alpha(\beta)\gamma_2$ heterotrimers or heterotetramers. The α -chains (FcR α) are type I integral membrane proteins and contain the Fc binding sites in their extracellular (N-terminal) region, whereas the β - and γ -chains exert mainly cytoplasmic functions, acting in cell signaling. (Fc γ RII is not associated with γ or β chains but contains its own cytoplasmic signaling sequence.) The ectodomains of the α -chains contain two (Fc γ RII, Fc γ RIII, Fc ϵ RI, and Fc α R) or three (Fc γ RI) Ig-like domains of ~ 80 amino acids with the C2 topology (Figure 1B) (see, however, Crystal and NMR Structures, below). The Ig-like domains are designated $\alpha 1$, $\alpha 2$, and $\alpha 3$ (Fc γ RI) reading from the N-terminus, with a high degree of homology between corresponding domains. These receptors contain a single transmembrane segment of ~ 20 amino acids and cytoplasmic sequences of varying length or, in the case of Fc γ RIIIB, a glycosylphosphatidylinositol anchor.

Fc ϵ RII/CD23 and Its Coreceptor, CD21

Unlike Fc ϵ RI and the other FcR, the low-affinity IgE receptor, Fc ϵ RII/CD23, is not a member of the Ig superfamily. CD23 (as this receptor is better known) is a type II integral membrane protein with a C-type (calcium-dependent) lectin domain at the distal, C-terminal end of the extracellular sequence (Figure 1C). It is thus related to the C-type lectin superfamily, which includes several adhesion molecules and carbohydrate pattern recognition receptors (13, 14).

The lectin domain is separated from the cell membrane by a heptad hydrophobic repeating sequence, which forms a three-stranded, 15-nm-long α -helical

coiled-coil stalk (sometimes called a “leucine zipper”). A C-terminal “tail” extends from the lectin domain. Two CD23 sequences, CD23a and CD23b, differ by seven and six amino acids, respectively, in their cytoplasmic N-termini and contain different signaling motifs that modify their functions (see Multiple Functions of CD23, below). CD23a is expressed in antigen-activated B cells, whereas CD23b expression is induced in a wide range of cells by IL-4. Messenger RNAs (mRNAs) for CD23a and CD23b are transcribed from different promoters in the same gene and the short first exons are spliced to the common coding sequence.

The lectin domain and/or tail contains the binding sites for all known ligands of CD23 including IgE, complement receptors CR2, CR3, and CR4 (also termed CD21, CD18-CD11b, and CD18-CD11c, respectively), and vitronectin. Calcium ions must be lodged in the conserved carbohydrate recognition sites to maintain the native fold of the lectin domain and for recognition of carbohydrate substituents in the complement receptors. CD23 recognizes only protein epitopes in IgE. The three-dimensional structure of the CD23 lectin domain has been modeled on the known structures of homologous proteins (15, 16) (see Figure 4A).

CD23 exists in the cell membrane as an equilibrium mixture of a 45-kDa monomer and a trimer; the formation of trimers leads to a 10-fold higher affinity for IgE (17, 18). Trimeric CD23 is cleaved at a specific site in the stalk to release a soluble fragment (sCD23) of 37 kDa, containing the lectin domain with an intact tail and a large part of the stalk. Further proteolysis of sCD23 at specific sites yields a relatively stable 16-kDa fragment, comprising the lectin domain and the proximal part of the C-terminal tail (see Multiple Functions of CD23, and Specification of IgE, below).

CD21 (coreceptor for CD23) plays a role in B cell survival and the specification of IgE (see Multiple Functions of CD23, and Specification of IgE, below). CD21 is a type I integral membrane protein containing in its extracellular sequence 15–16 domains homologous to the short consensus repeat, found in several other proteins of the complement cascade (Figure 1C). The other ligands for CD21 are C3d (and other fragments of the C3 component of complement), Epstein Barr virus, and α -interferon. Crystal structures have been determined for a fragment containing the two N-terminal domains of CD21 and its complex with C3d (19).

PHYSICAL PROPERTIES OF IgE

Shape and Flexibility of IgE

The image of a bent IgE molecule that has dominated the literature for over 10 years was originally advanced by Baird, Holowka, and co-workers, based on fluorescence energy transfer experiments (20). Donor and acceptor probes were attached to the two ends of a chimeric IgE, which bound Fc ϵ RI with the full affinity. The distance between the C-terminus and the antigen-combining site emerged as 71 Å, compared with the 175 Å expected for a planar Y-shaped structure modeled on

IgG1 (21). IgE was thus inferred to be highly bent. The distances between these fluorophores, located in the antigen combining site or the C-terminus, and the cell membrane were found to be greater than 100 Å or 50 Å, respectively. To satisfy these dimensions the receptor must also be bent in its complex with IgE on the cell membrane.

IgE was depicted as smoothly curved in the linker regions between Cε1 and Cε4, but the distance data were insufficient to define with any precision the angles between the domains. X-ray and neutron scattering profiles corresponded to a compact structure, compatible with only a few configurations, some of which could be eliminated by reference to other data. The best fit was a model in which the local 2-fold of the Cε3-4 domains is perpendicular to that of the Cε2 domains (22). The bend between Cε2 and Cε3 is more acute in the crystal structure of Fcε than in the models deduced from the solution studies (see Crystal and NMR Structures, below), which may mean that the Cε2-3 linker is flexible and that the crystal structure represents one extreme of the angular range available to the junction between the Cε2 and Cε3 domains. The solution data in this case would reflect the mean of a distribution of conformations (23). Fluorescence anisotropy decay measurements showed that IgE is less flexible than IgG in solution, and still less so in the Fcε-FcεRI complex (23).

IgM, the only other mammalian Ig possessing a domain homologous to Cε2, forms a pentamer, linked by a J chain and disulfide bonds. Pentameric IgM is planar as judged by electron microscopy (24) and X-ray scattering (25). Surprisingly, it forms a table-like structure when it binds to a multivalent antigen, the top formed by the Cμ3-4 region of the pentamer and the legs by the Cμ2-Fab elements, attached to the multivalent antigen (24). Thus the 90° angle between Cμ2 and Cμ3 reproduces the orientation of the corresponding domains of IgE.

Interaction with Receptors

The complex between IgE or Fcε and FcεRI is bimolecular (26). It is characterized by an association constant of K_a of 10^{10} M^{-1} , contrasting with 10^5 M^{-1} for IgG1 binding to FcγRIII (27) and 10^8 M^{-1} for IgG1 binding to FcγRI (11). This exceptionally high affinity is mainly a reflection of the very slow dissociation rate with a half-life of about 20 h for both IgE and Fcε on the receptor (28). The residence time on mast cells in tissues is further extended to ~14 days by restricted diffusion and rebinding to cell receptors (29, 29a).

The binding of IgE to CD23 on the cell exhibits two phases corresponding to affinities of $2-7 \times 10^7 \text{ M}^{-1}$ and $2-7 \times 10^6 \text{ M}^{-1}$ (for murine CD23). These are interpreted in terms of a monomer-trimer equilibrium (17). Two molecules of the monomeric 16-kDa CD23 fragment bind to the Cε3-4 fragment of IgE with similar affinities ($K_a \sim 10^5 \text{ M}^{-1}$) but different thermodynamic parameters (30). Accordingly, one IgE molecule may crosslink two molecules of the membrane-bound CD23 trimers, and several IgE molecules may form linear or cyclic oligomers.

CRYSTAL AND NMR STRUCTURES

From Models to Structures

Models for the three-dimensional structures of IgE-Fc (Fc ϵ), Fc ϵ RI α , and CD23 have been constructed based on homologies with proteins of known structure (1). Although crystal structures of Fc γ and IgGs have been available for some years, the lack of suitable crystals has long impeded determination of the Fc ϵ , FcR α , and both Fc ϵ :FcR α and Fc γ :Fc γ R structures. Recently these problems have been overcome, and high-resolution structures for Fc ϵ RI, Fc γ RII, and FcR γ III α -chains, C ϵ 3-4, Fc ϵ , and the two complexes, C ϵ 3-4:Fc ϵ RI α and Fc γ :Fc γ RIII α , have been obtained (Table 1). We give below a summary of these results and their significance.

C ϵ 3-4

The structure of this Fc subfragment (31) shows, as predicted (32, 33), that the C ϵ 3 and C ϵ 4 domains resemble C γ 2 and C γ 3 of IgG (21). Like Fc γ structures, the molecule also exhibits twofold (dyad) symmetry (Figure 2A). The angle between the C ϵ 3 domains is more acute than that between the C γ 2 domains;

TABLE 1 Crystal structures of IgE and IgG antibodies and their Fc receptor complexes

Structure	Protein data bank code	Resolution (Å)	Reference
Fc ϵ	1LS0	2.60	45
C ϵ 3-4	1FP5	2.30	31
Fc ϵ RI α	1F2Q	2.40	35
Fc ϵ RI α	1J86-9	3.20–4.10	36
C ϵ 3-4/Fc ϵ RI α	1F6A	3.50	37
Fc γ 1	1FC1	2.90	21
IgG1	1HZH	2.7	276
IgG1*	1IGY	3.20	277
IgG2a*	1IGT	2.80	278
Fc γ RII α	1H9V	3.00	279
Fc γ RII α	1FCG	2.00	38
Fc γ RII β	2FCB	1.74	39
Fc γ RIII β	1E4J	2.50	41
Fc γ RIII β	1FNL	1.80	40
Fc γ 1/Fc γ RIII β	1E4K	3.20	41
Fc γ 1/Fc γ RIII β	1IIS, 1IIX	3.00, 3.50	43

*murine protein.

Cε3-4 thus has a more compact structure than Fcγ. This difference results from a displacement propagated from a “hinge” within Cε3, not between Cε3 and Cε4, as might be expected (31). Carbohydrate chains attached to the pair of conserved asparagine residues (N394 in Cε3) mask hydrophobic residues corresponding to those protected by carbohydrate in Fcγ (at N297 in Cγ2).

FcεRIα

Since the review of this structure by Garman and co-workers (34, 35) more details have been brought to light by X-ray results of other crystal forms (36), including its structure in the complex with Cε3-4 (37). The extracellular region of FcεRIα contains two Ig-like domains, α1 and α2; these form an arch that fits into the binding site in IgE (Figure 2B). Contact residues on the receptor are located in α2 and the linker region between α1 and α2. All the FcRα structures that have been determined (Table 1) are broadly similar both in terms of their three-dimensional structure (34, 36, 38–40) and their mode of interaction with their cognate Fc domains (37, 41–43). Comparison of the several crystal forms of FcεRIα revealed one region of substantial structural variability, the segment of chain connecting the C and E strands in α2. At one extreme it takes the same form as a C' strand alongside the C strand in the conventional C2-type topology, and at the other it flips across to the other β-sheet to make hydrogen bonds with the E strand, in a manner similar to the D strand in C-type domains (Figure 1B).

Cε3-4:FcεRIα

The crystal structure of Cε3-4:FcεRIα revealed a 1:1 complex, in which the receptor engages with both Cε2-3 linker regions and both Cα3 domains (Figure 2B) (37, 44). Two structural features explain the 1:1 stoichiometry of the Cε3-4:FcεRI complex. First, the binding of FcεRIα at the pseudo-dyad axis between the two Cε3 domains obstructs the entry of a second receptor molecule. Second, induced conformational distortion leading to local asymmetry at the contact region disrupts the arrangement of residues in the receptor-binding site on the other face of the Cε3-4.

The two contact sites, one on each Cε3 domain, are quite different. The two chains and the sites they contain have been designated 1 and 2 (37) but A and B in the homologous Fcγ:FcγRIII complexes (41, 43). Here we refer to site 1 in chain A and site 2 in chain B, following Wan et al. (45). Formation of the high-affinity Cε3-4:FcεRIα complex occludes a contact surface of 1830 Å², 860 Å² at site 1 and 970 Å² at site 2 (37). The residues involved are shown in Figure 3A, from which it can be seen that sites 1 and 2 on IgE are made up of partly overlapping sets of contact residues. Of the 15 FcεRIα contact residues, 7 are aromatic and, of these, 5 are surface-exposed tryptophans. The large number of aromatic side chains and large occluded surface area undoubtedly contribute to the exceptionally high stability of the complex. In site 1, Y131 of the receptor projects into a pocket in the Fcε formed by the BC and FG loops of Cε3 and the

C ϵ 2-3 linker, while in site 2, P426 from C ϵ 3 intercalates between receptor residues W87 and W110, forming a "proline sandwich." This interaction is conserved in the Fc γ 1:Fc γ RIII α complex and across all the Fc γ 1:Fc γ RIII complexes (Figure 3A).

The contact residues in sites 1 and 2 are only partially accessible in the uncomplexed C ϵ 3-4 (31). Thus, formation of the complex clearly demands a conformational change in the C ϵ 3-4 polypeptide, involving separation of the C ϵ 3 domains, by movement of the "hinge" within C ϵ 3. This may explain why mutations in the region of the hinge (e.g., in the AB loop) affect Fc ϵ RI binding even though they are far from the receptor in the complex (46).

Fc ϵ

The crystal structure of Fc ϵ (45) exposed the C ϵ 2 domain pair to view for the first time. The C ϵ 2 domain pair is folded back asymmetrically onto the C ϵ 3 and C ϵ 4 domains in an acutely bent configuration (Figure 2C,D). This both bears out the earlier evidence for bent IgE and Fc ϵ (20, 22) and accounts for the interaction seen in solution between C ϵ 2 monomers and C ϵ 3-4 (28).

The C ϵ 2 domains display a novel interdomain interface, quite different from predictions based upon other C domain pairs (32, 33). In contrast to the typical C interface as between C ϵ 4:C ϵ 4, which is predominantly hydrophobic (2326 Å², 63% nonpolar), the C ϵ 2:C ϵ 2 interface is much smaller and mainly hydrophilic (1760 Å², 25% nonpolar), with nine trapped water molecules. The C ϵ 2 domains (C ϵ 2A and C ϵ 2B) are linked by two interchain disulfide bonds between cysteine residues C241 and C328. The configuration of these bonds is of interest because they were first modeled as crossed (C241A-C328B, C241B-C328A) (33) and later parallel (C241A-C241B and C328A-C328B) to conform to a biochemical analysis (32). They are in fact crossed in the Fc ϵ crystal structure (Figure 2C,D). The C ϵ 3-4 construct used for the crystal structure analyses (31, 44) contained only C328 and is linked by a C328-C328 bridge. The flexibility of the linker region, however, may allow the two C ϵ 3 domains freedom to adopt their preferred relative orientation.

The bent Fc ϵ results in an asymmetric disposition of the C ϵ 2 domains. C ϵ 2 of chain B packs against the C ϵ 3 domain of chain A and even makes contacts with the C ϵ 4 domain of this chain (Figure 2C,D). C ϵ 2A, however, makes few contacts with C ϵ 3B and none with either C ϵ 4 domain. These interactions distort the symmetry of the C ϵ 3 domains, resulting in a structure that is more asymmetric with respect to these domains than the C ϵ 3-4 fragment in either the free or receptor-bound state. Figure 3B compares the C ϵ 3 and C ϵ 4 domains of Fc ϵ with the isolated C ϵ 3-4. The figure shows that C ϵ 3A (against which C ϵ 2B packs) adopts the more or less "closed" conformation seen in uncomplexed C ϵ 3-4, whereas that of C ϵ 3B is close to the "open" configuration of C ϵ 3-4 in its complex. As a result, in the free Fc ϵ structure, only site 2 in chain B is available for receptor binding.

Thus, the free receptor molecule can dock onto site 2 on C ϵ 3B of Fc ϵ but not site 1, as seen in Figure 4B. In fact, for this to happen the free receptor structure with its CE connection in the α 2 domain must exist as a D rather than a C' strand (see above) to avoid steric interference from C ϵ 3A. For the

receptor to dock on site 1 also, and bind in the same way as in the Cε3-4:FcεRIα complex, not only must the Cε3A domain be in the open state, but the D strand of the CE region of the receptor α2 domain must adopt the C' strand conformation beside strand C. Because the change in Cε3A requires a displacement of Cε2B, the structure of Fcε implies that a conformational change in the Cε2 domains must also accompany receptor binding.

Cε2:FcεRIα

The conformation of the monomeric Cε2 domain in solution has been solved by NMR spectroscopy (28). Disulfide bridging was precluded by mutations C241S and C328S. The monomer did not self-associate even at a concentration of 3 mM, probably because of the relatively hydrophilic interdomain interface. It did, however, show a weak interaction ($K_d \sim 200 \mu\text{M}$) with FcεRIα, the first indication of a direct interaction with Cε2. The contact residues are found on a patch of the Cε2 sequence (Figure 4Bi). In the modeled complex (45), with the receptor attached at site 2 in Fcε, these residues make no contact with FcεRIα (Figure 4Bi), but movement of the Cε2 domains away from Cε3-4 (shown schematically in Figure 4Bii) would bring them into apposition and allow the Cε3A domain to adopt the open conformation required for receptor binding at site 1.

The Cε2 domains in Fcε and IgE have a significant influence on the kinetics and affinity of the interaction. Cε3-4 fragments bind to the receptor with faster on- and off-rates than Fcε and IgE and a 10-fold lower affinity ($K_a = 1.6 \times 10^9 \text{ M}^{-1}$ vs. $2.7 \times 10^8 \text{ M}^{-1}$, respectively) (28). Additional contacts with the receptor and the (slow) conformational change may account for the slow dissociation of IgE from the complex. Furthermore, initial contact at site 2 (depicted in Figure 4Bi), followed by the conformational change to permit contact at site 1, affords a structural explanation for the reported biphasic nature of the binding kinetics (46–48).

Comparison of IgG-FcR and IgE-FcεRI Interactions

The structures of the IgE- and IgG-receptor complexes are quite similar. Both are 1:1 complexes and therefore differ from the 2:1 stoichiometry of the other Ig receptor interactions, including those with FcRn, C1q, bacterial Ig-binding proteins A and G, and rheumatoid factors in which the receptor binds to symmetric sites on opposite sides of Fcγ (49). CD23 also displays a 2:1 stoichiometry of binding to Cε3-4 (30). The effective univalency of the Ig is a safeguard against the catastrophic eventuality of receptor crosslinking by single Ig molecules with consequent activation of cells in the absence of antigen. Such protection is a fundamental requirement of the adaptive immune system.

Common features in the manner of Fc-FcRα binding extend to the strands and loops that engage between receptor and ligand. Contact residues at site 2 (Figure 3A), which are mainly hydrophobic, are more extensively conserved. It has been suggested that the greater variation in site 1 provides a “fine-tuning” of affinities for the different classes and subclasses of antibody (44).

EFFECTOR FUNCTIONS OF FcεRI

Allergic Disease

The acute early phase of the allergic reaction reflects the actions of mast cell mediators, some of which are preformed and stored in cytoplasmic granules, awaiting release upon allergen provocation, whereas others result from the activation of enzymes. The well-known products of activated mast cells include histamine, serotonin, lipid mediators (prostaglandins, leukotrienes), proteases (tryptase, chymase), chemokines (eotaxin, RANTES), and cytokines. Among the latter are TNF- α , GM-CSF, macrophage inflammatory protein-1 α , and a number of "T helper (Th) 2-cell type" cytokines, such as interleukins IL-3, IL-4, IL-5, IL-6, IL-9, IL-10, and IL-13.

The activities of allergen-activated mast cells are said to "orchestrate the allergic response" (50). The local effects (e.g., in skin in atopic dermatitis or eczema) include (a) enhanced local vascular permeability, leading to leakage of plasma protein, such as fibrinogen (resulting in local deposition of crosslinked fibrin and tissue swelling); (b) increased cutaneous blood flow, with intravascular trapping of red cells, owing to arteriolar dilation; (c) increased loss of intravascular fluid from postcapillary venules to produce erythema (giving the name of IgE); (d) and other effects such as itching, owing to stimulation of cutaneous sensory nerves by histamines (3, 51, 52).

Local variations on this theme characterize allergic reactions in other allergic diseases, resulting in different signs and symptoms (53, 54). Chronic asthma has additional complications associated with remodeling of airway tissues, notably smooth muscle hypertrophy and mucus cell hyperplasia, to cause persistent wheezing and congestion.

IL-4 released by mast cells upregulates VLA-4 on the local epithelium, which leads to the recruitment of VCAM-1-expressing T cells, basophils, eosinophils, and monocytes to sites of allergen provocation. IL-3 is an autocrine growth factor for mast cells and basophils. Eotaxin and RANTES attract T cells and eosinophils into the tissue, where the latter proliferate in response to IL-5. In turn these cells generate more inflammatory mediators to create a cascade of inflammatory reactions (the "mast cell-leukocyte-cytokine cascade") manifested in the late phase of the allergic response.

Allergen activation of mucosal mast cells may also result in the enhancement of local IgE synthesis. Crosslinking of FcεRI leads to the expression of CD40-ligand (CD40-L), which may foster interaction with local cells expressing CD40, notably dendritic cells and B cells. Contact with resident B cells and secretion of IL-4 and IL-13 induces class switching to IgE (50, 55) and may constitute a positive feedback mechanism for local IgE synthesis (Figure 5) (see H-Chain Class Switching to IgE, and Compartmentation, below).

Immediate hypersensitivity is classically associated with IgE, FcεRI, and mast cells in mucosal tissues. In situ assays reveal that FcεRI is copiously expressed on

mucosal mast cells in the nose and lung and is further upregulated in subjects with allergic rhinitis and asthma (56). Fc ϵ RI is upregulated by IgE and IL-4 (57–69). The elevated expression of Fc ϵ RI on tissue mast cells may therefore be secondary to the enrichment of the tissues in IgE and IL-4 compared with the circulation.

Fc ϵ RI is upregulated in part by IgE, which prevents protease digestion at the cell surface (63, 70), and in part by IL-4, which may act through STAT6 sites in the α -chain promoter (D. Fear, unpublished results). Monomeric IgE binding to Fc ϵ RI has a further unanticipated effect: It promotes the survival of the cells (71–73a). The accretion of new mast cells from the circulation and local proliferation, the increased longevity of these cells, and the upregulation of Fc ϵ RI all contribute to the abundance of this receptor in tissues. The effector function of mast cells is significantly enhanced, because they undergo degranulation at lower concentrations of antigen and release larger amounts of preformed mediators, lipid products, and cytokines in response to IgE-mediated activation (57, 59, 63, 67, 69).

Association with the γ -chain dimer is required for Fc ϵ RI and Fc γ RIII to be expressed on the surface of mast cells. The quantity of γ -chain is limiting in mast cells, and consequently the enhancement of Fc ϵ RI expression by IgE and IL-4 is accompanied by suppression of Fc γ RIII expression; it follows that IgG antibody-dependent anaphylaxis is also suppressed (74). Fc ϵ RI signaling pathways and mechanisms of mast cell activation have been intensively studied and comprehensively reviewed (75). The pathophysiological effects of the individual mast cell mediators in allergic disease are also well known (53, 54).

Allergic Disease: Is IgE Essential?

This question has been addressed in two recent studies of mouse models, both of which conclude that IgG is just as effective, if not more so, in triggering anaphylactic reactions. In the first of these studies systemic anaphylaxis in Fc ϵ RI α -deficient mice was induced by a single intraperitoneal injection of ovalbumin, followed three weeks later by a second injection of the antigen (76). Mice lacking Fc ϵ RI α were no less susceptible than wild-type mice. Passive immunization with anti-DNP IgG1 and challenge with DNP-haptenated human serum albumin (DNP-HSA) yielded similar results, except that mast cell degranulation was not observed in either the Fc ϵ RI α -deficient or wild-type mice.

In the second study the reactions of mice deficient in IgE, Fc ϵ RI, mast cells, Fc γ RII/III, or macrophages were compared with wild-type mice after a single intravenous or intraperitoneal injection of goat antimouse IgG (GaMIgG) and challenge with goat IgG two weeks later (77). IgE, Fc ϵ RI, or mast cell ablation had no effect, whereas Fc γ RII/III or macrophage ablation prevented systemic anaphylaxis. In this system anaphylaxis was clearly engendered by IgG activation of macrophages via Fc γ RIII (Fc γ II being unable to function in this manner). Platelet-activating factor was identified as the vasoactive mediator.

Whereas these studies contribute to an understanding of the fundamental biology of IgG and IgE antibody classes in mouse, they do not directly bear on

the pathogenesis of allergic disease in man: In the first place, they focus on the response to a single antigen challenge at the minimal time after primary immunization, a procedure that bears little relationship to the usual manner in which allergic responses are elicited. The results in fact are those of a primary immune response. Further, Fc ϵ RI is not expressed in effector cells other than mast cells and basophils in mouse (75); nor is CD23 expressed on cells other than B cells and follicular dendritic cells. Mouse, unlike human, IgE binds with low affinity to IgG receptors (75), and mouse, unlike human, CD23 does not interact at all with endogenous CD21 (78).

Fc ϵ RI-deficient mice are even less analogous to humans, who are not in general deficient in expression of this receptor (all too often quite the contrary). Whereas Fc γ RIII may be under-expressed in allergen-sensitive tissues, in consequence of the over-expression of Fc ϵ RI on the mast cells, Fc γ RIII is likely to be over-expressed in the Fc ϵ RI α -deficient compared with wild-type mice, allowing abnormal function of IgG in anaphylaxis.

The conclusion must be that IgE, Fc ϵ RI, and mast cells or basophils are not essential for active or passive systemic anaphylaxis in the absence of Fc ϵ RI, an unnatural condition in mammals. All three are required for anaphylactic reactions in tissues and in particular those of the target organs of allergic disease.

Improved Animal Models and Protocols for the Study of Allergic Disease

Several approaches have been used to improve the mouse models of allergic disease. In one study using wild-type mice, antigens were delivered by aerosol to simulate the route of allergen entry into the respiratory tract in man. In a landmark paper Gelfand and co-workers showed that nebulized interferon- γ (IFN- γ) inhibits the development of primary and secondary allergic responses in mice immunized against ovalbumin through the airway (79). The effect could be ascribed to the generation of Th1 cells, which directed class switching to IgG2a instead of IgE (see H-Chain Class Switching to IgE, below). This treatment averted the danger of airway hyper-responsiveness and the sensitization of cutaneous mast cells against ovalbumin.

Two lessons can be drawn from this work: (a) Together with similar results obtained with two other inhibitors, soluble IL-4 receptor (80) and an anti-IL-5 antibody (81), it shows that direct delivery of inhibitors to the site of sensitization suppresses not only the primary but also the more important secondary allergic response. (b) Topical treatments acting at more than one point in the inflammatory cascade, whether by IFN- γ or soluble IL-4R (which result in Th1 cell polarization; see H-Chain Class Switching to IgE, below) or neutralization of IL-5 (required for the activation of eosinophils) may restore tolerance to allergens.

Humanization of Fc ϵ RI α (82, 83) in a transgenic mouse (hFc ϵ RI α) may also render the mouse model more relevant, as demonstrated in a study of intestinal bowel disease, resembling Crohn's disease in humans (84). hFc ϵ RI α mice express Fc ϵ RI with the same cell-type distribution as humans. It was recently shown that

FcεRI is expressed in rat with the same cellular distribution as in humans (85). This probably explains why the rat has often proved a better model than the mouse for human allergic disease. The use of airway immunization and hFcεRI mice or genetically modified rats should prove advantageous in future studies of allergic disease.

Eosinophils, Platelets, and Monocytes

A hallmark of allergic disease is the infiltration of the tissues with increased numbers of eosinophils (86). Eosinophils express FcεRI, but most of the protein is confined to the cytoplasm (87–89); there is little evidence for IgE-dependent function. The migration of eosinophils to the sites of allergen provocation is orchestrated by Th2 cytokines. They are recruited to the tissues by adhesion of VLA-4 to VCAM-1, upregulated on the local endothelium by IL-4, and by eotaxin, which binds the chemokine CCR3, almost exclusively expressed by eosinophils and basophils. IL-5 promotes their survival and activation in the tissue. The relatively eosinophil-specific basic proteins, which are stored in granules, are major basic protein, eosinophil cationic protein, eosinophil peroxidase, and eosinophil-derived neurotoxins, all of which are toxic to the bronchial epithelial cells and helminthic parasites. Lipid bodies are the most prominent source of leukotrienes. Cytokines, such as GM-CSF, act in an autocrine manner to prolong survival. The activated cells undergo “piecemeal degranulation” (as do basophils) without cell death, but vigorous allergen stimulation induces cytolysis. In the pathophysiology of asthma, eosinophils may promote cough and airway wall remodeling.

One of the well-documented IgE-dependent activities of eosinophils (90) and platelets (91) is killing of schistosome parasites (92) [see Parasitic Disease (Schistosomiasis), below]. Monocytes are well-known IgG effector cells in antibody-dependent cell-mediated cytotoxicity; we have recently shown that they can also act in IgE antibody-dependent phagocyte-mediated killing of tumor cells (93).

The mean number of surface-expressed FcεRI molecules in monocytes from normal (nonatopic) individuals is ~3000 per cell (93), compared with 200,000 for blood basophils (60, 61). Levels of FcεRI of expression on monocytes are very heterogeneous, and only a minority of the cells express FcεRI detectable by flow cytometry (93, 94). A higher proportion of HLA-DR⁺ peripheral-blood dendritic cells express FcεRI than monocytes, as reflected in their relative activities in antigen presentation (95) (see Function of FcεRI on Antigen-Presenting Cells, below). Degranulation of eosinophils may be required to release the stored FcεRI.

As in mast cells and basophils, FcεRI is upregulated on monocytes (96, 97), cord blood-derived dendritic/Langerhans cells (98), and eosinophils (99, 100) by incubation with either IgE or IL-4. Accordingly, somewhat higher levels of expression of the receptor on monocytes were observed in peripheral blood monocytes in atopic individuals (96) and in the lung mucosa of atopic and nonatopic asthma patients (56) compared with healthy nonatopic individuals.

FcεRI is expressed in monocytes as an αγ₂ trimer. Transfection of a monocytic cell line with an expression vector for the β-chain increased the level of expression

by 5–7-fold. The β -chain enhances the strength of signal transduction (101) and assists in transporting the receptor to the cell surface (102). Neither incubation of the cells with IgE or IL-4, nor expression of the β -chain in a monocytic cell line, elevated expression to the level seen in mast cells and basophils. The basis, then, of the differences in expression between cell types remains uncertain, although upregulation by IgE and IL-4 may occur in the manner indicated. The lower expression may relate to the primary function in antigen presentation, while at the same time limiting IgE effector functions to minimize damage to host tissues.

Parasitic Disease (Schistosomiasis)

In all animal models and in humans elevated IgE concentrations, including IgE specific for schistosomes, and blood and tissue eosinophilia are hallmarks of schistosomiasis (103). A specific IgE antibody was protective against schistosomiasis in rats, and adoptive transfer experiments revealed that eosinophils or platelets bearing cytophilic IgE were crucial (104). Epidemiological studies of endemic helminthic worm infections in humans have demonstrated a correlation between the serum IgE concentration and resistance to infection (105).

Experiments in mice in which either the IgE ϵ -chain or Fc ϵ RI α -chain gene were deleted, however, only partially supported an IgE protective response. IgE-deficient mice exhibited increased worm burdens and reduced granulomatous inflammation in liver following primary infection with *Schistosoma mansoni* (106); this is consistent with a role for IgE in host protection. However, mice without Fc ϵ RI α suffered normal worm burdens and increased egg granuloma formation, arguing against a role for Fc ϵ RI in host resistance (107). The ambiguous results may be due to some of the same problems that confound the use of mouse models of allergic disease (see Allergic Disease: Is IgE Essential?, above).

It has been suggested that the IgE-Fc ϵ RI-mast cell complex constitutes only one of many IL-4- and IL-13-mediated effector mechanisms in mammals and that these cytokines in turn are the agents of only one of a number of strategies that have evolved for immune defense against extracellular parasites (108). Different strategies may then be effective against different parasites, and some may be ineffective or even harmful. This diversity may broaden the defensive options against a range of parasites large enough to defy antigenic recognition. Accordingly, IL-13 (though not IL-4), IL-4R α , and STAT6 were each required for expulsion of the gastrointestinal nematode parasite *Nippostrongylus brasiliensis* in mice (109). Allergic disease may be the legacy of this primitive strategy.

FUNCTION OF Fc ϵ RI ON ANTIGEN-PRESENTING CELLS

Most data regarding Fc ϵ RI activity come from studies of this receptor in the “professional” antigen-presenting cells (APC), monocytes, dendritic cells, and Langerhans cells, although mast cells (110) and eosinophils (92, 111) expressing Fc ϵ RI are also capable of presenting antigens to T helper cells (Figure 5).

The addition of allergen-specific IgE to monocytes depleted of their endogenous IgE brings about a hundred- or thousand-fold increase in efficacy of antigen presentation to Th cells (112). Peripheral blood dendritic cells, however, not only express FcεRI at higher levels than monocytes but also prime Th cells with 5–10 times greater efficiency: Far fewer dendritic cells are required to attain the same response elicited by monocytes (95).

The biological consequences of IgE antibody-dependent antigen presentation have been cogently summarized in two recent reviews (113, 114). The IgE dependence of antigen presentation implies that only immune responses to allergen, previously mediated by IgE antibodies, will be amplified. In the case of dendritic cells, antigen presentation may also widen the spectrum of allergen epitopes recognized by IgE, because these cells can present antigens to naive Th cells. The predicted effect is a lowering of the threshold allergen concentration for subsequent responses. A similar argument pertains to CD23-facilitated antigen presentation (115) (see next section). Dendritic cells migrate from sites of antigen uptake to local lymphoid tissue for presentation of antigen to Th cells; their activity is therefore strictly regional.

APC are a potent source of cytokines (e.g., IL-1, macrophage inflammatory protein-1α, TNF-α) and eicosanoids. The crosslinking of FcεRI on dendritic cells by allergens may therefore modulate or even initiate allergic inflammatory responses in allergen-exposed tissues. For example, IL-1 and TNF-α upregulate VCAM-1 and ICAM-1 on endothelial cells and cause transmigration of inflammatory cells into inflamed tissue. Thus, APC can contribute to the inflammatory cascade when tissues of allergic individuals are exposed to allergen.

MULTIPLE FUNCTIONS OF CD23

Cleavage by Proteases

The initial cleavage of the membrane-associated CD23 stalk to generate the largest (37-kDa) soluble fragment (sCD23) from the 45-kDa parent polypeptide chain is effected by a membrane-bound metalloprotease (116). Other proteases attack sCD23 at specific sites in the residual stalk sequence, terminating in formation of the 16-kDa fragment, which is also the product of digestion by the house dust mite, *Dermatophagoides pteronyssinus*, Der p I protease (116a) (see H-Chain Class Switching to IgE, below). CD23 and 16-kDa sCD23 may have opposite effects on the regulation of IgE synthesis (13, 117).

Facilitated Antigen Presentation

CD23a facilitates antigen presentation in murine and human B cells in vitro and in murine B cells in vivo (Figure 5). CD23 in human B cells mediates IgE-dependent Der p II allergen presentation to autologous Der p II-specific T cell clones in vitro (118, 119). CD23 is bound in the membrane of human B cells to HLA-DR, with

which it undergoes endocytosis and recycling (120). The association may facilitate the transfer of peptides to the HLA-DR in peptide-loading compartments of the cell and also (after recycling to the cell surface) adherence of B cells to T cells during antigen presentation. Antibodies against either CD23 or CD21, the presumptive ligand on allergen-activated Th cells (121), inhibit antigen presentation (122). Because antigen presentation by CD23 is isotype rather than antigen specific and delivers the antigens attached to IgE, it will stimulate mainly immune responses to allergens (115). In the Th2 microenvironment of mucosal tissues B cells switch to IgE and thereby amplify the preexisting IgE response. Thus, IgE-dependent CD23-mediated antigen presentation to Th cells may exercise positive feedback control (Figure 5B).

Feedback Regulation of IgE Synthesis

CD23 also acts as a buffer in negative feedback regulation of IgE synthesis. CD23 knock-out mice overexpress IgE, whereas transgenic mice overexpressing CD23 are deficient in IgE (123, 124). In isolated B cells crosslinking of CD23 or IgE and CD23 resulted in the downregulation of IgE synthesis; thus, mCD23 and/or proteins associated with mCD23 may deliver signals to the B cell to inhibit IgE synthesis when IgE concentrations are of the same order as K_d ($\sim 0.1 \mu\text{M}$). Negative feedback regulation would thus occur in a much higher concentration range than that required for sensitization of mast cells and basophils ($K_d \sim 0.1 \text{ nM}$), when positive feedback mechanisms are dominant (13).

Murine IgE binds with low affinity to $\text{Fc}\gamma\text{RII}$, which contains an inhibitory motif (75); thus, murine IgE synthesis is inhibited when IgE concentrations approach the K_d of this interaction (125, 126). Human IgE does not bind to IgG receptors but operates by way of an isotype-specific mechanism of feedback control through CD23.

Function in IgE Transport

Studies in a rat model of food allergy showed that CD23 expressed by enterocytes transports IgE-antigen complexes, formed in the intestinal lumen, across the epithelium to the underlying tissue, where the antigen stimulates local hypersensitivity reactions (127). This activity probably relies on IgE antibodies released from the tissue into the lumen and returned to the tissue after antigen capture. Local CD23 and MHC class II expression are dramatically increased in diseases of the gastrointestinal tract associated with elevated serum IgE levels (128); this argues for the activity of CD23, as well as $\text{Fc}\epsilon\text{RI}$, in regional antigen presentation (see Facilitated Antigen Presentation, above).

Involvement in the Germinal Center Reaction

A gradient of CD23 expression that increases with distance from the centroblast compartment is seen on follicular dendritic cells [immune complex-presenting cells in germinal centers (GC) of the secondary lymphoid organs; see H-Chain

Class Switching to IgE, below) (128a)]. It is likely that CD23 rescues selected B cells from apoptosis by interacting with CD21 on their surface (129, 130). In a model of the GC reaction binding of CD23 to CD21 on peripheral blood B cells lowers the concentration range in which surrogate antigen (anti-IgM), on coligation of IgM, stimulates B cell proliferation (13, 131).

The Function of CD23 Fragments

sCD23 promotes differentiation of GC B cells into plasma cells in vitro (129), presumably interacting with CD21 and thus extending their lifetime and capacity to proliferate, until they receive further signals (see next section). CD23 also promotes the growth and differentiation of promyelocytes into basophils and prothymocytes into T cells. CD23 fragments of 25 kDa or larger (and similarly anti-CD21 antibodies) stimulate IgE synthesis in B cells on incubation with suboptimal concentrations of IL-4 and anti-CD40 (133, 134); as before, this may allow the greater proliferation required for class switching to IgE. CD21 and IgE epitopes on CD23 are separate, but their binding affinities are of the same order ($K_d \sim 0.1 \mu\text{M}$). Accordingly, trimeric sCD23 clusters mIgE and CD21 coexpressed in the cell membrane (135). This implies that CD23 can stimulate proliferation of IgE-committed plasmablasts by coligating mIgE and CD21 in the cell membrane prior to terminal differentiation and contribute to IgE expression in mucosal tissues [(1, 13) and see Local Regulation of IgE Synthesis, below].

H-CHAIN CLASS SWITCHING TO IgE

Immunoglobulin Gene Organization and Class Switching

The expressed immunoglobulin L chain gene is assembled from one each of multiple $V\kappa$ and $J\kappa$ gene segments and the $C\kappa$ gene segment on chromosome 2p11.12 or the corresponding λ gene segments on chromosome 22q11.2. The H-chain gene is assembled from VH , diversity (D), and H-chain joining (JH) gene segments and the $C\mu$ gene segment on chromosome 14q32 (136) (Figure 6). Immature (naive) B cells, expressing mIgM ($C\mu$), migrate from the bone marrow into the circulation, where they may encounter antigen to initiate somatic hypermutation (SHM), followed by clonal selection for antigen affinity (or apoptosis in the absence of selection) in GC of the secondary lymphoid organs. This is accompanied by H-chain class switching; the cells then develop into memory B cells or Ig-secreting plasma cells before returning to the circulation (G Kelsoe, unpublished results). Plasma cells migrate to the bone marrow and generate a continuous supply of antibodies. These provide the first line of defense in subsequent immune responses and participate in affinity maturation by mediating the presentation of antigen to B cells by follicular dendritic cells in the GCs (128a). Whereas secondary lymphoid organs are the normal sites of SHM and class switching, these processes may also occur at novel sites of chronic inflammation, e.g., in autoimmune and allergic diseases (see Local Regulation of IgE, below).

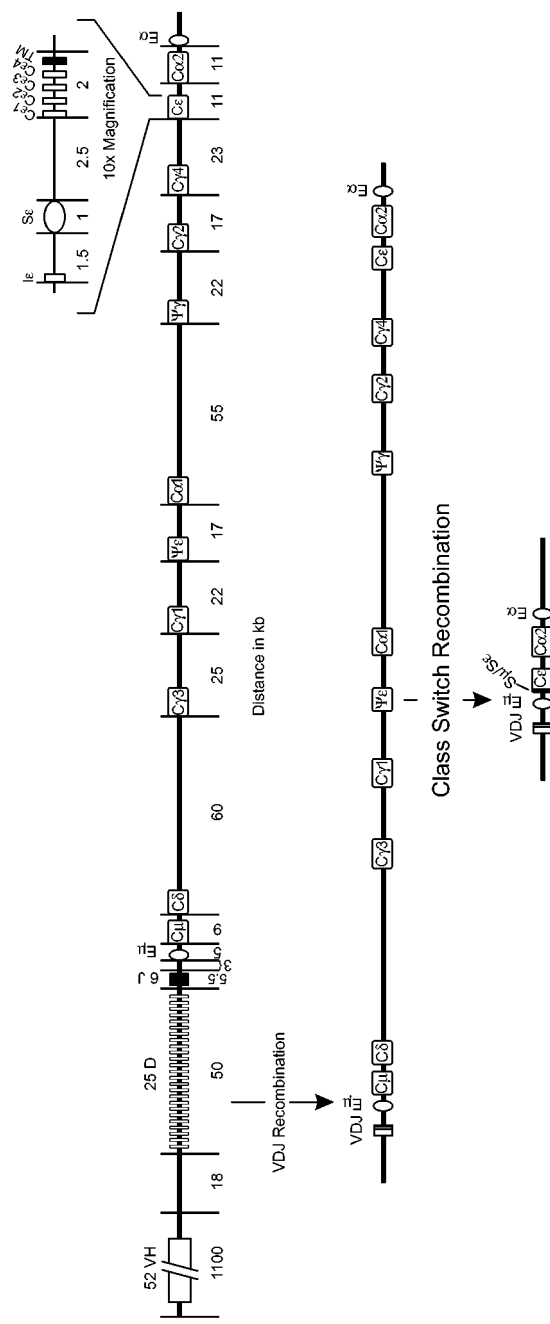


Figure 6 Scale diagram of the human heavy chain locus, showing VDJ recombination and class switch recombination to IgE.

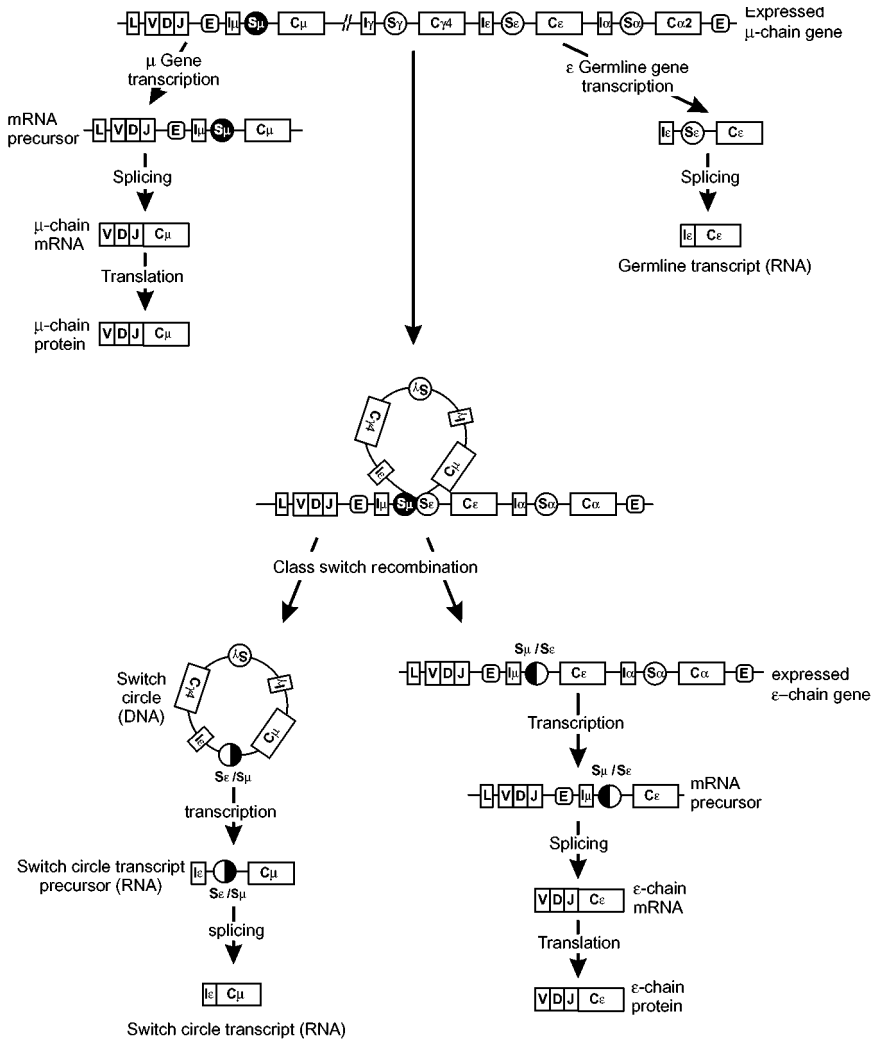


Figure 7 Mechanism of IgE class switch recombination.

Class switching, required for the production of IgG, IgA, and IgE, involves somatic recombination between C_μ and one of eight CH genes in a tandem array downstream of C_μ on chromosome 14q32 (136) (Figure 6). The intervening sequence is looped out and deleted from the genome (Figure 7). The rearranged gene is expressed from the nearby VH promoter under the influence of the accompanying intronic enhancer (E μ). (IgD is expressed by differential splicing of an mRNA precursor containing both C_μ and C δ .)

The array of homologous CH genes evidently arose by gene duplication and diversification. Sequence analysis suggests that the C_ν gene (encoding the ν

H-chain of IgY) in a cold-blooded ancestor was duplicated and evolved into the C γ and C ϵ genes of mammals. The selective value may have been functional specialization, with IgG performing immune surveillance in the circulation and IgE in solid tissues in warm-blood mammals (139).

The Role of T Helper Cells

B cells require help from Th cells to mount an effective immune response to T cell–dependent antigens (140). Two Th cell subsets, Th1 and Th2, derived from Th0 cells, play a central role in isotype determination through cognate interaction with B cells and the secretion of specific cytokines. Th1 cells secrete IL-2 and IFN- γ , whereas Th2 cells secrete IL-3, IL-4, IL-5, IL-9, IL-10, IL-13, and GM-CSF. IL-4 and IL-13 specify class switching to IgE. The other Th2 cytokines contribute to the wider functions of these cells in immune defense against parasites and in the pathogenesis of allergic disease (108, 141–145) (see Effector Functions of Fc ϵ RI, above).

Th1 and Th2 cells differentiate from precursor cells when activated through their T-cell receptor/CD3 complexes by processed antigen/class II MHC on APC (Figure 8). The signaling pathway for Th1 cell differentiation involves IL-12, interferon- γ (IFN- γ), STAT4, STAT1, and the transcription factor, T-box, expressed in T cells (T-bet). IL-12 is required for the activation of STAT4, which then induces IFN- γ gene expression via T-bet. The resulting IFN- γ (through STAT1) forms part of an autoregulatory circuit with T-bet, whereby IFN- γ stimulates T-bet gene expression and vice versa (146, 147). The IFN- γ secreted by the Th1 cells directs switching to IgGs but not to IgE in B cells. IFN- γ also antagonizes the differentiation of Th0 to Th2 cells, thus reinforcing the original lineage decision.

Th2 cell fate is governed by the transcription factors GATA-3 and c-maf; GATA-3 results in the production of the signature cytokines IL-4, IL-5, IL-9, and IL-13. GATA-3 autoregulates its own promoter and appears to control the accessibility of the IL-4 gene for transcription. IL-4 stimulates class switching to IgG4 and IgE and also suppresses IFN- γ gene expression; consequently IFN- γ and IL-4 reassert their own lineage decision by positive and negative feedback mechanisms.

Th cell polarization is now understood in terms of cell division and IFN- γ and IL-4 chromatin remodeling. When Th0 cells are activated they begin to produce IL-2 immediately, but the synthesis of IFN- γ or IL-4 is linked to cell division (148–150). The frequency of IFN- γ gene expression in the cell population increases through successive cell cycles, whereas IL-4 expression sets in only after three cell divisions. The concerted effects of cycling and cytokine signaling relieve epigenetic repression by demethylation and hyperacetylation of chromatin in the region of the genes (149, 151–154).

APC, notably activated macrophages, and a subset of dendritic cells (DC1; see next section) specialize in the production of IL-12. The nature of the APC is therefore important because, in the absence of IL-12, activated Th cells generate IL-4, which downregulates IFN- γ and hence IL-12. This may explain why B cells, acting as APC (155–157) (see Figure 5), and dendritic cells that fail to produce

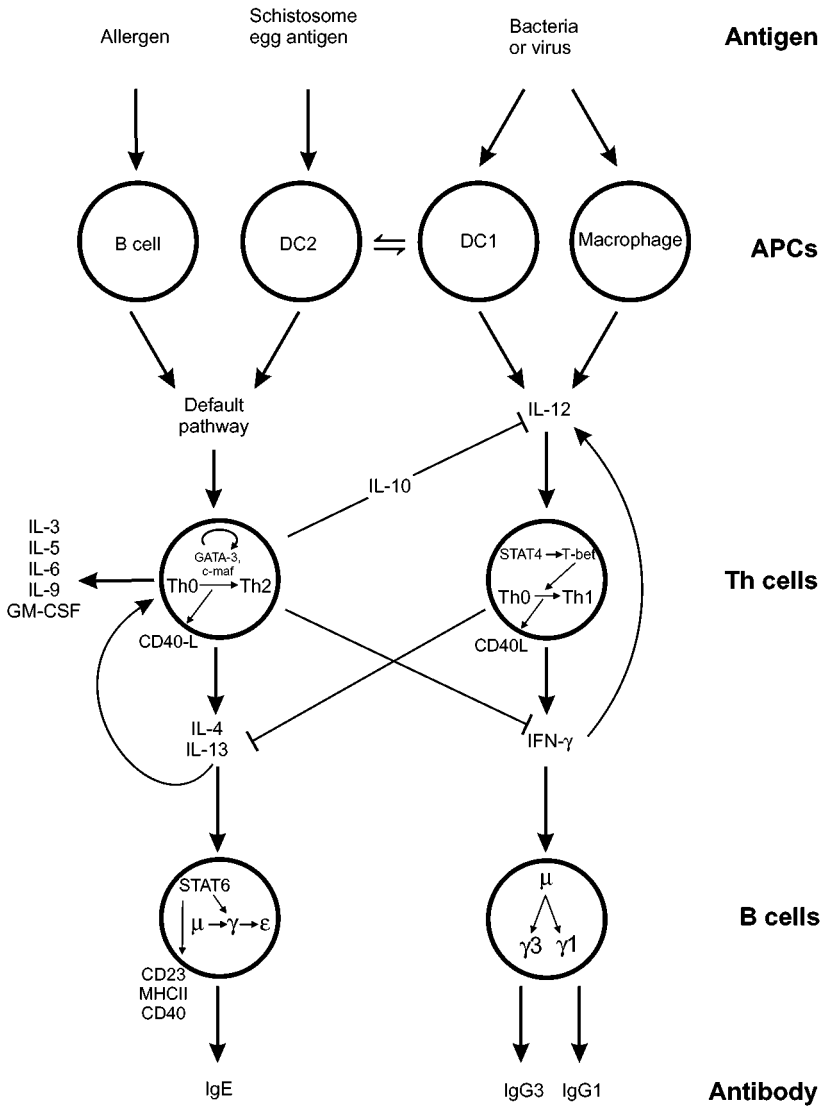


Figure 8 Factors that specify IgE and IgG.

IL-12 induce Th2 responses by default. IL-4 secreted by activated mast cells (158) and basophils (159) may contribute to Th2 cell differentiation in mucosal tissues, e.g., the respiratory tract of atopic asthmatics. Exposure to aeroallergens would then force Th0 cells in the mucosa to differentiate into IL-4-secreting Th2 cells; the autoregulatory circuit would thereafter maintain the Th2 state in these cells and induce the differentiation of newly recruited Th0 into Th2 cells.

The Role of Dendritic Cells

Whether dendritic cells direct Th cells into the Th1 or the Th2 pathway may be pre-determined by signals received from the antigen and the microenvironment (160). Bacterial constituents, such as lipopolysaccharide and oligonucleotides with CpG motifs, induce the production of IL-12 and IL-18 in mouse DC1 and macrophages. Certain microbes, *Bordetella pertussis* toxin and poly(rI:C), the double-stranded viral RNA analogue, induce DC1 (corresponding to Th1) polarization, whereas extracellular *Vibrio cholerae* toxin and extracts from schistosomal egg antigens induce DC2 polarization (161). A glycolipid (LDN-DF) was recently identified as a schistosomal egg constituent, which may act at the host-parasite interface, perhaps triggering this innate immune response (162). The relevant receptors and signaling pathways remain to be elucidated.

The locally acting agents responsible for DC2 differentiation may be prostaglandin E₂ (PGE₂) and IL-10 (163, 164), which are known to suppress IL-12 production in dendritic cells (164a). Other factors, such as the antigen (type, dose), the host (genetic background, age, prior infection history), and the environment (natural adjuvants) may all influence whether a dendritic cell becomes DC1 or DC2 (165–168).

Rat respiratory dendritic cells and murine Peyer's patch dendritic cells elicit Th2 responses (164a), whereas splenic dendritic cells engender Th1 responses (170). Viral antigens can induce Th2-committed (CD11c[−]) pre-dendritic cell maturation into dendritic cells that elicit IFN- γ -producing T cells (171). This shows the plasticity of dendritic cell function determined by antigens.

The Role of Allergens

There is no simple answer to the question, "What makes an antigen an allergen?" As noted above, there may be structural components recognized by the innate immune system. For some antigens, the route of entry (the microenvironment) or particle size, which in the case of aeroallergens affects the extent of penetration into the respiratory tract, may be overriding factors. Both the glycolipid structure and location in the lung mucosa may account for the propensity of the schistosomal egg antigen (LDN-DF) to induce an IgE response, although not all of the IgE is directed against the parasite. In addition, adjuvants in the environment (e.g., pollutants) or in vaccination protocols may influence the direction of class switching.

Many common allergens, not least Der p I, the fecal antigen of the house dust mite, are proteases. Der p I attacks two known substrates at specific sites, namely IL-2R (CD25) and CD23, which may affect the immunological outcome (Th1 versus Th2). Inactivation of Der p I protease activity increases IgE synthesis in vivo (172) and in B cells incubated with T cells and IL-4 in vitro (173). Treatment of T cells with Der p I enhances their ability to stimulate IgE upon reconstitution with B cells in vitro (174). Accordingly, Der p I conditions T cells to produce more IL-4 and less IFN- γ , which could clearly contribute to dust mite stimulation

of IgE responses. Der p I also cleaves membrane-bound CD23 to release a 16-kDa fragment (116a, 176), which may upregulate IgE synthesis by eliminating feedback control (see Multiple Functions of CD23, above).

Genes and the Environment

No description of the mechanisms of Th cell polarization would be complete without mention of the more global effects of genetic polymorphisms and environmental risk factors. No less than half of the susceptibility to asthma is determined by genetic predisposition (177), but attempts to identify the responsible genes have often led to conflicting answers owing to their multiplicity (178–180). Not surprisingly various “candidate” genes, e.g., the IL-4, IL-4R α -chain, IL-9, IL-13, STAT6, and CD14 genes, determine the effective Th1/Th2 cell balance. CD14 is the myeloid pattern-recognition receptor for lipopolysaccharide in APC, which leads to Th1 cell polarization (181, 182). Other candidate genes, e.g., the Fc ϵ RI β and mast cell chymase genes, are related to effector mechanisms (178).

The fetus is exposed to a Th2 environment in the uterus, and the immune system is therefore Th2-polarized at birth. It is believed that immune deviation normally occurs by the induction of Th1 responses and the resulting suppression of Th2 responses following bacterial infections in the neonate. This may be averted, especially in genetically predisposed individuals, by the high standards of hygiene in industrialized countries (the “hygiene hypothesis”) and overexposure to allergenic substances such as the fecal antigens of the house dust mite or fungal spores in the atmosphere. These conditions are thought to have contributed to the dramatic rise in the prevalence of allergic disease in recent decades (143, 178).

Mechanism of Class Switching to IgE

Class switching proceeds directly or sequentially to downstream CH genes from C μ to C γ , C α , or C ϵ . Each class switching event occurs in three distinct stages: germline gene transcription, class switch recombination (CSR), and B cell differentiation into Ig-secreting plasma cells (183). As an example we describe direct class switching from IgM to IgE (Figure 7).

The μ and ϵ germline genes are transcribed from their intervening (I) exon promoters. RNA polymerase traverses the I exon, switch (S) region, and CH exons and stops at the normal (mRNA) polyA addition site of the μ chain and ϵ chain germline gene, and I μ is spliced to C μ , and I ϵ to C ϵ . The I exons contain stop codons in all three frames and hence these transcripts are said to be “sterile.” The chromosomal DNA is cleaved in the two switch regions (S μ and S ϵ), and the ends of the chromosome and those of the excised intervening DNA fragment are joined to make the rearranged chromosome and resultant “switch circle” DNA. The rearranged ϵ gene can then be transcribed into the ϵ chain mRNA precursor from the V region promoter, stimulated by the intronic enhancer (E μ), and the initial

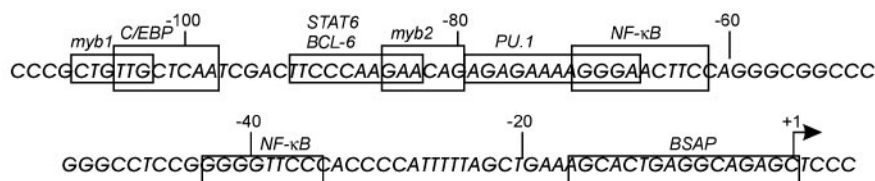
transcript is spliced to yield mRNA encoding either the membrane or secreted form of the ϵ chain. The mRNAs are then translated into protein, assembled from the H- and L-chains and either transported to the cell membrane (mIgE) or, after B cell differentiation, secreted (IgE).

Class switching is coupled to cell division and is therefore a slow process: Class switching to IgE can be induced by incubation of Th cells (or a combination of IL-4 and anti-CD40 or soluble CD40-L) with B cells in vitro. In vitro germline gene transcription is evident after a few hours, and CSR and mIgE expression at around day 6, followed by B cell differentiation and IgE secretion after day 10 (184). This is comparable to the time required for antibody generation in the primary immune response in vivo.

ϵ Germline Gene Transcription

IL-4 or IL-13 bind to the common α -chain of the IL-4 (IL-4R) and IL-13 (IL-13R) receptors to activate ϵ germline gene transcription in B cells (185). The signal transduction pathway is well understood (186). First the receptor is phosphorylated by the associated JAK1 and JAK3 kinases. The phosphorylated receptor recruits STAT6 from the cytoplasm, and the activated JAKS phosphorylate STAT6. Phosphorylated STAT6 molecules migrate to the cell nucleus and bind to response elements in a number of genes, including one in the germline gene promoter (Figure 9).

A



B

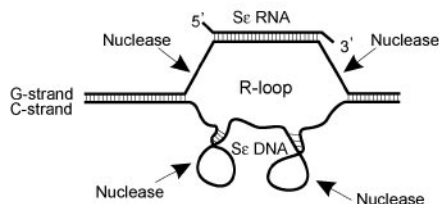


Figure 9 (A) Transcription factor binding sites on the human ϵ -germline gene promoter. (B) Structure of an R-loop formed during germline gene transcription.

Figure 9A shows the more complete constellation of transcription factors controlling transcription of the human ε germline gene (187, 188). They include NF- κ B, PU-1, BSAP, and C/EBP and are counteracted by negatively acting factors, Myb, and possibly BCL-6, which must be displaced before transcription can proceed. Crosslinking of CD40 on B cells activates NF- κ B and enhances ε germline gene transcription (189). Unstimulated NF- κ B exists in a complex with an inhibitor ($\text{I}\kappa\text{B}$), in the cytoplasm. When phosphorylated by IKK1 and IKK2, $\text{I}\kappa\text{B}$ is degraded and a nuclear localization sequence in NF- κ B is unmasked. This allows the transcription factor to migrate from the cytoplasm to the nucleus and bind to the accessible response elements in many genes, in addition to the ε germline gene. Many NF- κ B target genes belong to a wider family of inflammatory response genes (190). PU-1 is associated with differentiation of a common lymphoid/myeloid cell precursor into the lymphocyte lineage and BSAP with the differentiation of a common T/B cell precursor into B cells (191).

Recent evidence suggests that Myb inhibits ε germline gene transcription, probably by blocking access to the 3' side of the STAT6 site (192) (see Figure 9). Such a factor or combination of factors may determine the probability of initiation of ε germline gene transcription in a stochastic manner (see Specification of IgE, below).

Class Switch Recombination

The mechanism of CSR (the term confined to the stage of DNA recombination in class switching) proceeds by four sequential steps: (a) selection of the target S region, (b) recognition of the DNA target sequence or structure, (c) DNA cleavage, and (d) repair and ligation (193, 194).

S regions are located between the I exons and CH1 exons of each CH gene (except C δ) in the germline DNA (Figure 7). They contain multiple pentameric (GAGCT and GGGGT) repeats and longer repeats extending over a total length of ~ 1 –10 kb; S ε is relatively short, comprising only 1–2 kb. Recent evidence suggests that the essential property of the S sequences for CSR is their capacity to form hairpin loops by palindromic S sequences when the DNA is unwound for transcription (195, 196). Hairpin formation in the noncoding strand may be facilitated by the failure of the nascent RNA to dissociate from the coding strand, thus generating an "R loop" (197) (see Figure 9B). DNA breaks may be introduced at the exposed junctions between single and double-stranded regions in the hairpins and at the ends of the RNA-DNA hybrid.

If, as expected from the R-loop model, some of the products of cleavage have staggered ends, with little complementarity to those of potential S partners in CSR, the sequences would have to be modified before DNA ligation. Microhomologies have been detected at switch junctions, suggesting that base pairing may nucleate the formation of the hybrid S junction and allow the entry of repair enzymes (198). Error-prone polymerase(s) are implicated by the occurrence of point mutations and insertions in the S junctions, whereas deletions from the germline DNA sequence are assumed to reflect the activities of exonuclease(s).

Elements of the MRN DNA repair complex, Mre11, Rad50 or 51, and Nbs1, may be involved in the CSR mechanism. Nbs-1 stimulates the unwinding of DNA duplexes and hairpin cleavage, whereas Rad50 is responsible for ATP-binding and joining of the noncomplementary ends (199). This complex, together with phosphorylated histone, γ -H2AX, is involved in repairing lesions in DNA caused by X-rays (200). Two components of the MRN complex, Nbs1 and γ -H2AX, colocalize with the H-chain gene locus during CSR; others were not detected (201).

Ku70/Ku80 and DNA-protein kinase are required for CSR, thus implicating the nonhomologous end-joining pathway in the mechanism. The Ku70/Ku80 complex binds to single- or double-stranded DNA ends and recruits DNA-protein kinase to such sites. It is not clear whether additional mechanisms help the broken DNA ends to locate each other. One suggestion is that alternative splicing, whereby I exons are joined to a distal CH1 exon, brings the associated DNA S regions into closer proximity (figure 1 in Reference 194).

In B cells class switching occurs after several rounds of cell division, and the H-chain gene locus is replicated from a single origin downstream of the 3' enhancer (E α in Figure 6) early in the S phase of the cell cycle in B cells (202). Class switching occurs at the G1/S stage of the cell cycle (203). What happens when the replication fork and RNA polymerase, travelling in opposite directions, meet on the DNA? Does this clean the slate for the resumption of transcription at G1/S of the next cycle or bypass the transcription complexes? This is unknown, and there is little information in general about such situations.

The enzyme activation-induced cytidine deaminase (AID) is essential for both CSR and SHM (193, 204, 205). AID is homologous to APOBEC-1, the catalytic subunit of an RNA-editing complex, which deaminates C to U, thereby introducing a stop codon in the mRNA coding for apolipoprotein B, resulting in a truncated protein. AID itself exhibits cytidine deaminase activity *in vitro*. AID expression is B cell-specific and particularly highly expressed in GC and during the G1/S stage of the cell cycle, in accordance with the spatial and temporal requirements for activity in CSR (203). Ectopic expression of AID is sufficient to activate CSR in hybridomas (206) and fibroblasts (207). This implies that all other participating proteins are either constitutively expressed or induced by AID. AID is not necessary for germline gene transcription or DNA cleavage (208, 209). Candidate targets for AID activity therefore include the DNA itself or mRNA encoding enzymes that act in DNA repair or B cell survival during CSR (210). The identification of the AID substrate(s) is now a matter of priority. (See Note Added in Proof.)

B Cell Differentiation

Immediately after CSR the rearranged gene is expressed and mIg is exported to the cell surface. The differentiation of memory B cells into Ig-secreting plasma cells entails gross changes in cell phenotype and function. The cells lose their motility and responsiveness to many external signals. The nucleus contracts in response

to the shutdown of previous functions and develops an extensive endoplasmic reticulum for Ig synthesis and secretion; a plasma cell secretes up to 0.1 ng Ig per day, up to 50% of total protein synthesis (211).

How antigen triggers differentiation of B cells into plasma cells is now understood in broad outline (212, 213): Signals transmitted through the α and β signaling subunits of the receptor stimulate phosphorylation by mitogen-activated protein kinase, leading to the degradation of BCL-6 (214). The function of BCL-6 here is to repress the gene for B lymphocyte-induced maturation protein 1 (Blimp-1) (215, 216); thus, the degradation of Bcl-6 allows Blimp-1 expression. Blimp-1, in association with hGroucho and class I histone deacetylases, inhibits transcription of genes coding for the mIg α -chain, Myc, BSAP, STAT6, AID, Ku70/80, DNA-protein kinase, and other proteins involved in the processes occurring in the plasma cell precursors. BSAP represses the expression of X-box binding protein (XBP-1), required for terminal differentiation, so the lack of BSAP removes a further block to differentiation. Terminal differentiation is reinforced by the inhibition of renewed BCL-6 by Blimp-1, which appears following degradation of BCL-6.

Differentiation to memory cells occurs through IL-10, secreted by Th2 cells, which upregulates CD27 in GC, allowing interaction with CD70⁺ T cells and B cells (217). In response to IL-4 and CD40 crosslinking, CD27⁺ B cells proliferate and may thus undergo class switching to IgE (218, 219). CD40 signalling downregulates the Blimp-1 gene (220), restraining this differentiation, whereas CD23 signalling through CD21 upregulates BCL-2 to rescue the B cells from apoptosis (129). Together, CD40 and CD23 signaling promote cell proliferation and B cell survival, thus increasing the probability of class switching to IgE. CD23 may also act in the preferential selection of IgE-committed B cells (see Multiple Functions of CD23, above).

Specification of IgE

How does IL-4 specify IgE? The prevailing view is that it acts by facilitating transcription of the ϵ germline gene, coupled to local unfolding of chromatin, much as has been convincingly demonstrated for the IFN- γ and IL-4 genes in specifying the Th1 and Th2 lineages (see The Role of T Helper Cells, above). Transcription of the ϵ germline gene was said to be accompanied by silencing of γ genes (e.g., γ 2b in mouse) (221). The "chromatin accessibility model" for the direction of class switching, equating transcription with chromatin accessibility (see Figure 2 in Reference 194), was built on this supposition. However, the chromatin structure of the CH genes has never been investigated. Developmental stage-specific unfolding of the murine κ and λ L-chain genes, in fact, precedes gene transcription (222).

The established fact is that IL-4 and IL-13 are the only cytokines that stimulate ϵ germline gene transcription and (with CD40 signaling) class switching to IgE in both murine and human B cells. At the same time, IL-4 and CD40-L stimulate

transcription and CSR of all the other germline genes in human B cells, leading to CSR and secretion of the corresponding antibody classes (223, 224). In the latter study by Cerutti and co-workers the chosen mixture of cytokines contained not only IL-4 and anti-CD40 but also IL-10 and IL-6, which are known to stimulate B cell differentiation (225, 226). Another group found that IL-10, together with anti-CD40, stimulated class switching to IgGs but not to IgE, whereas IL-4 stimulated CSR to IgGs and IgE, though only IgE was secreted (227).

Recent work in our laboratory has shown that the majority of mature, naive (IgD⁺) B cells exhibit constitutive transcription of one or more germline genes, including γ and ε genes, and that IL-4 and anti-CD40 upregulate the level of transcription (D. Fear et al., N. McCloskey, G. Felsenfeld, H.J. Gould, unpublished results). IL-4 and IL-13 are the only cytokines capable of upregulating ε germline gene transcription to approximately the same level as that of the γ germline genes. It has been suggested that a threshold level of transcription may be necessary, if not sufficient, for CSR (228).

IL-4 acts by way of STAT6 sites, which are found in all the γ germline genes, as well as in the ε germline gene. If (as the best evidence indicates) all the genes are equally accessible, then the original question can be reformulated: How can the γ germline gene transcription be upregulated in the absence of IL-4?

Hodgkin and co-workers have demonstrated that class switching to several isotypes, including IgE, in murine B cells is tightly coupled to the number of cell divisions. Switching to IgG1 occurs after three cell divisions and to IgE after five (229–232). This can be rationalized, even if the mechanism cannot be defined, by postulating an isotype-specific probability of switching at each round of replication. This could depend, for instance, on the density of nascent germline gene transcripts (see ε Germline Gene Transcription, above). The highest probability of switching to a given isotype varied with the IL-4 concentration, but the number of cell cycles at the peak was constant. Some cells underwent apoptosis before arriving at the required number of cell divisions for class switching to IgE, and more than half of the remainder failed to make the switch.

Sequential switching to downstream isotypes is a feature of both murine (233–235) and human (236–242) B cells. Thus, the lag in class switching to IgE may result in part from the sequential process. However, it was observed that the probabilities of switching from IgM or Ig2b to IgE were identical, suggesting there may be an inherent delay in switching to IgE, the mechanism of which is not yet understood.

During sequential switching the I exons corresponding to previously expressed germline genes are excised from the genome and only transiently transcribed from the resulting switch circles (243) (see Figure 7). The possibility of detecting germline gene transcripts from the intermediate isotypes must decrease with time. This may account for the observation that γ 2b transcripts decline while ε transcripts increase between 3 and 5 h in murine B cells stimulated with lipopolysaccharide and IL-4 (221), the cornerstone of the chromatin accessibility model.

SOMATIC HYPERMUTATION OF IgE VH REGIONS

Somatic hypermutation (SHM) is characterized by the accumulation of point mutations across rearranged V region DNA, particularly at "hotspots" such as RGYW and its reverse complement WRCY (where R = A or G, Y = C or T, W = A or T). These hotspots occur more frequently in complementarity-determining regions (CDR) than framework regions (FR), making the CDRs especially susceptible to mutation (244). A single mutation can engender a 10-fold increase in antibody affinity (245). The ratio of replacement (R) relative to silent (S) mutations may reveal evidence of antigen selection. In the absence of antigen selection R/S ratios are generally twice as high in CDR as FR, reflecting the relative frequency of RGYW motifs and the imprint of antigen selection (246).

Unusual patterns of R/S ratios and VH usage have been found in the V regions from IgE H-chain (VH-C ϵ) sequences from allergic individuals. VH5 is one of the smallest VH gene families containing two members out of the total of 52 functional VH genes (247). In patients with allergic rhinitis (248), allergic asthma (249–251), and atopic dermatitis (252) VH5 is greatly overrepresented in IgE, particularly in the target organs compared with peripheral blood B cells. In the nasal mucosa of patients with allergic rhinitis about one fifth of VH-C ϵ sequences were VH5, compared with about half that number in peripheral blood B cells from the same group of individuals (248). The bias was even more pronounced in a patient with severe asthma, one third of whose VH-C ϵ sequences were VH5 (251). A similar proportion of VH5-C ϵ sequences were detected in IgE⁺ B cells from the spleen of asthmatic patients (249), whereas the overall abundance of VH5 in the splenic B cells of the combined antibody classes was only 8%. Two explanations have been suggested: Either common allergens or autoallergens, perhaps acting as superantigens, recognize FR, particularly FR3 (249), or FR3 imposes a favorable structure on the CDRs (253).

Somatic mutations in the VH-C ϵ sequences of the allergic subjects peak in the CDRs and occur at approximately the expected frequency in hotspot motifs. Analysis of the R/S ratios, however, revealed certain anomalies. In the study of patients with atopic dermatitis the majority of VH5-C ϵ sequences exhibited R/S ratios well below 2.9 in CDR (252), and in the patients with allergic rhinitis R/S ratios in the mucosa were 1.5 in CDR and 2.7 in FR (248). These unusual R/S ratios could arise from the local activity of allergens recurrently or chronically stimulating the immune system, or from a superallergen, leaving the imprint of SHM in the FR.

Analysis of VH-C ϵ sequences in allergic individuals suggests the presence of clonally related but diverged families of B cells, as indicated by the presence of shared and unique mutations. These could emerge if the cells circulated repeatedly through germinal centers (GC), with consequent accumulation of somatic mutations and clonal selection, but several observations suggest that this is not the case. The occurrence of clonal families in mucosal tissues suggests that clonal expansion may occur locally (248, 251). The coexistence of unmutated (germline) and varying extents of somatically mutated VH-C ϵ sequences in asthmatic lung

mucosa points to local somatic mutation (251). The argument is based on the low probability of migration of an unmutated clone to a lymph node, there to undergo somatic mutation, before returning to the very same site. The presence of clonally related families comprising more than a single isotype (IgM, IgG, IgA, and IgE) in both the lung and nasal mucosa (248, 251) suggests by the same reasoning that both class switching and SHM occurred locally. These results and the observation of AID expression in the nasal mucosa of a patient with allergic rhinitis (248) provide supporting evidence for local class switching (see next section).

LOCAL REGULATION OF IgE

Local IgE Synthesis

Local synthesis of IgE has long been inferred on the grounds that IgE antibodies are found in the nasal secretions of subjects with allergic rhinitis and in bronchoalveolar lavage fluid from patients with allergic asthma. However, exudation from serum or active transport of IgE, as recently demonstrated for CD23 (127) (see Multiple Functions of CD23, above), could not be excluded. More persuasive evidence comes from the observation of antibodies in the secretions when they could not be detected in the serum by RAST assay or by skin prick test (9, 254). Recent work demonstrating *de novo* IgE synthesis in the nasal mucosa of subjects with allergic rhinitis and the lung mucosa of asthmatics has resolved this issue (255, 256). Local IgE synthesis significantly allows maintenance of the state of immediate hypersensitivity in the mucosal mast cells (see Compartmentation, below).

The source of IgE has therefore been determined, though not of that of the B cells. As class switching to IgE undoubtedly occurs in GC, the committed cells could have migrated from local lymphoid tissues through the circulation to the inflamed areas at sites of allergen provocation, but this is not easily reconciled with the presence of clonal families of different isotypes (see Somatic Hypermutation of IgE VH Regions, above). The notion of clonal expansion and selection within the mucosa is supported by the high proportion of CD19⁺ B cells containing ϵ chain mRNA in the nasal mucosa (a third and a tenth of the total number of cells in allergic rhinitis and healthy nonatopic control subjects, respectively) (257). The mRNA is evidently translated, for the proportions of B cells and plasma cells expressing mIgE in the nasal mucosa of allergic rhinitis patients are much the same (258). Only one in 10^4 – 10^3 of peripheral blood B cells are committed to IgE (6, 7), so there is a 100- to a 1000-fold enrichment of these cells in the mucosa.

Persistence of IgE Synthesis

The synthesis of IgE antibodies in the nasal mucosa of grass pollen-sensitive subjects continues outside the grass pollen season (255). How is the life of the IgE-secreting cells or clones extended to allow for a constant source of IgE without

allergen stimulation? IgE-expressing B cells may be effectively selected for longevity if they have undergone a greater number of cell divisions and escaped apoptosis prior to class switching (see H-Chain Class Switching to IgE, above). A large proportion of IgE-secreting plasma cells are exceptionally resistant to killing by X-irradiation (259, 260).

The survival of IgE-committed B cells may be promoted following the upregulation of CD23b by IL-4 and the release of sCD23; sCD23 can form a heterotrimer with mIgE and CD21 (135), and coligation of mIg and CD21 by CD23 stimulates B cell proliferation (13, 131) (see Multiple Functions of CD23, above). Long-lived IgG antibody-secreting plasma cells in bone marrow are nurtured by stromal cell factors (see H-Chain Class Switching to IgE, above). Recent studies of IgA regulation suggest that this antibody class may be generated in the gastrointestinal mucosa of mouse by Th cell-independent class switching (261) or even direct VDJ recombination with C α (262). Thus, different microenvironments evidently favor certain antibody classes over others.

Allergen Stimulation of Local Class Switching to IgE

Evidence of local class switching to IgE comes from observations of various transient markers for the dynamic process of class switching (see H-Chain Class Switching to IgE, above). Local allergen challenge stimulates the appearance of ϵ germline gene transcripts in B cells within 24 h (257). Local allergen challenge also stimulates the appearance of IL-4 mRNA in Th cells in the same interval, suggesting a causal relationship. IL-4 mRNA in Th cells and ϵ germline gene transcripts in B cells were also identified in the nasal mucosa of grass pollen-sensitive individuals during the grass pollen season (263). Allergen stimulation of IL-4 synthesis by Th cells, which in turn induce ϵ germline gene transcription in B cells, is thus again implied. More surprisingly ϵ -germline gene transcripts were constitutively expressed in the lungs of nonatopic asthmatics, suggesting that the IgE antibodies may be directed against autoantigens (264, 265).

To eliminate the possibility that the Th cells and B cells migrate from the circulation after mast cell activation of the tissue by allergen in the allergen challenge studies, explants from the nasal mucosa were incubated with allergen *ex vivo*. Again it was observed that IL-4 mRNA in Th cells and ϵ germline gene transcripts appeared in B cells after 24 h (266).

Although germline gene transcripts represent the first step in class switching, this is not to say that the process proceeds through the subsequent steps: CSR, B cell differentiation, and secretion of IgE (see H-Chain Class Switching to IgE, above). Switch circle DNA, transient markers of CSR, have been observed in the nasal secretions of allergic rhinitics challenged with ragweed allergen mixed with diesel exhaust particles (267), and in the nasal mucosa of allergic rhinitics exposed to allergen (P. Takhar, L. Smurthwaite, S.R. Durham, & H.J. Gould, unpublished results). These observations suggest that allergen stimulates CSR in the nasal mucosa of allergic rhinitics.

Analogy Between Autoimmune and Allergic Disease

Repeated or chronic stimulation of inflammatory responses in mammalian tissues may result in SHM and class switching in abnormal locations. This is well documented in rheumatoid arthritis (268) and insulin-dependent diabetes mellitus (269). Synovial B cells in rheumatoid arthritis are long-term memory cells and thus play a part in the chronic inflammatory reactions. The microenvironment of the synovium allows the activation of naive and memory B cells, diversification of their V gene repertoire, affinity maturation, and clonal expansion (268, 270). Synoviocytes support the differentiation of the activated B cells *in vitro* (271).

Chvatchko and coworkers found that germinal centers were formed in the lung after airway antigenic challenge in mouse and that this led to local IgE synthesis (272). Together with the cellular and molecular studies described above this provide compelling evidence of a similar development in allergic disease.

COMPARTMENTATION

Is the Respiratory Tract Mucosa Self-Sufficient in IgE?

The allergic state, characterized by immediate hypersensitivity, depends on the permanent sensitization of mast cells in the target organ. We have observed that IgE synthesis occurs between seasons in the nasal mucosa of grass pollen-sensitive patients with allergic rhinitis (see Local Regulation of IgE, above). We ask here whether this is sufficient to maintain the sensitization of mast cells or whether the main source of IgE is outside the tissue. Nasal B cells synthesize $\sim 4 \times 10^9$ molecules/day/mm³ biopsy [calculated from the data of Smurthwaite et al. (255)]. Immunohistochemical studies of sections of the tissue yield a count of ~ 5000 mast cells/mm³ nasal biopsy (S.R. Durham, personal communication). There are $\sim 2 \times 10^5$ receptor molecules per cell (69), and the half-life of IgE in skin is ~ 14 days (29, 29a, 273). Uninterrupted saturation of these receptors therefore requires the *de novo* synthesis and secretion of 4×10^7 molecules of IgE/day/mm³. We conclude that the rate of IgE secretion generously exceeds the minimum requirement for saturation.

The figures are notional, there are other IgE-binding cells in the tissue, and about half of the IgE is in any event nonspecific antibody (255), but maximum sensitization requires the occupancy of less than 10% of the FcεRI molecules on the mast cell (69). The excess unbound IgE presumably corresponds to that measured in secretions and in the serum, where it is diluted with IgE antibodies from other mucosal sources. This IgE may also suffice to maintain the perpetual sensitization of Langerhans cells in the epithelium. Upon exposure to allergens these cells would migrate to the local lymphoid tissue and initiate a GC reaction. This in turn would lead to a transient response, resulting in repopulation of the mucosa with memory Th cells following their release into the circulation and recruitment into the tissue. Scharenberg and Kinet refer to the more persistent IgE synthesis in tissues as “pump priming” (274).

The above calculation indicates that the duration of mast cell sensitization by IgE is a critical characteristic. IgG diffuses out of tissue much more quickly than IgE (29, 255, 273), a function both of the number of IgG receptors and the higher intrinsic dissociation rate of IgG from the receptors. The stability of the IgE-FcεRI complex is now understood in structural terms (45, 275) (see Crystal and NMR Structures, above). IgE antibody synthesis is also a critical factor; indeed it is a diagnostic feature of allergic rhinitis (29, 255, 257).

Coordination of the Local Response

Mast cells and basophils, along with IgE and IgE receptors, constitute a potent weapon against parasites but also threaten the host. A number of mechanisms are coordinated to maximize the former and minimize the latter activity by focusing the response on the affected tissue. IL-4 and other mast cell mediators induce selective recruitment of inflammatory cells, cell activation, and cell proliferation at the site of antigen provocation, resulting in escalation of the inflammatory response in the tissue. Eosinophils, stimulated by IL-5, play a particularly ambiguous role in host protection versus host damage (86).

The release of IL-4 and IL-13 and other Th2 cytokines by allergen-activated mast cells enhances the IgE response by stimulating Th2 cell differentiation, which results in class switching to IgE in B cells and increased production of IgE; this in turn protects and resensitizes FcεRI on the mast cells in the tissue in a positive feedback mechanism (green and blue arrows in Figure 5). Antigen presentation to Th2 cells may occur by dendritic cells expressing FcεRI, by B cells expressing CD23a, or directly by mast cells (black arrows in Figure 5A, B, and C, respectively). In any case the afferent IgE response to the allergen or parasite occurs no further away than the local lymphoid tissue. The local Th1/Th2, IgE/IgG, and FcεRI/FcγR cell fate decisions are all mutually antagonistic (Figure 8). STAT6 plays an important role in the coordination of Th2 responses. The regulation of IgE and the affinity of IgE for FcεRI are combined to exclude IgG, which would not confer immune protection of such long duration as IgE antibodies, from the tissue. Local Th2 cell-mediated immunity operates in a coordinated fashion in expelling parasites and initiating the cascade of early- and late-phase allergic reactions.

SUMMARY

The reaction of IgE with its receptors is central to the phenomenon of allergy. X-ray crystallography and NMR have recently yielded detailed information about the structures of these molecules and their interactions. Together with the outcome of work on the equilibrium and dynamic characteristics of the antibody-receptor interactions, considerations of the spatial and temporal factors of IgE regulation and effector functions enable us to assemble a relatively complete picture of the mechanisms involved in the allergic response. This will undoubtedly open new avenues for therapeutic intervention in allergic disease.

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NOTE ADDED IN PROOF

Recent studies on the effects of UNG uracil glycolase deficiencies in chicken B cells and $ung^{-/-}$ mice (282, 283) provide further evidence that SHM and CSR involve dC $\rightarrow$ dU deamination by AID and suggest that abasic sites may be targets for an apyrimidic nuclease and generation of adjacent mutations by an error-prone DNA polymerase.

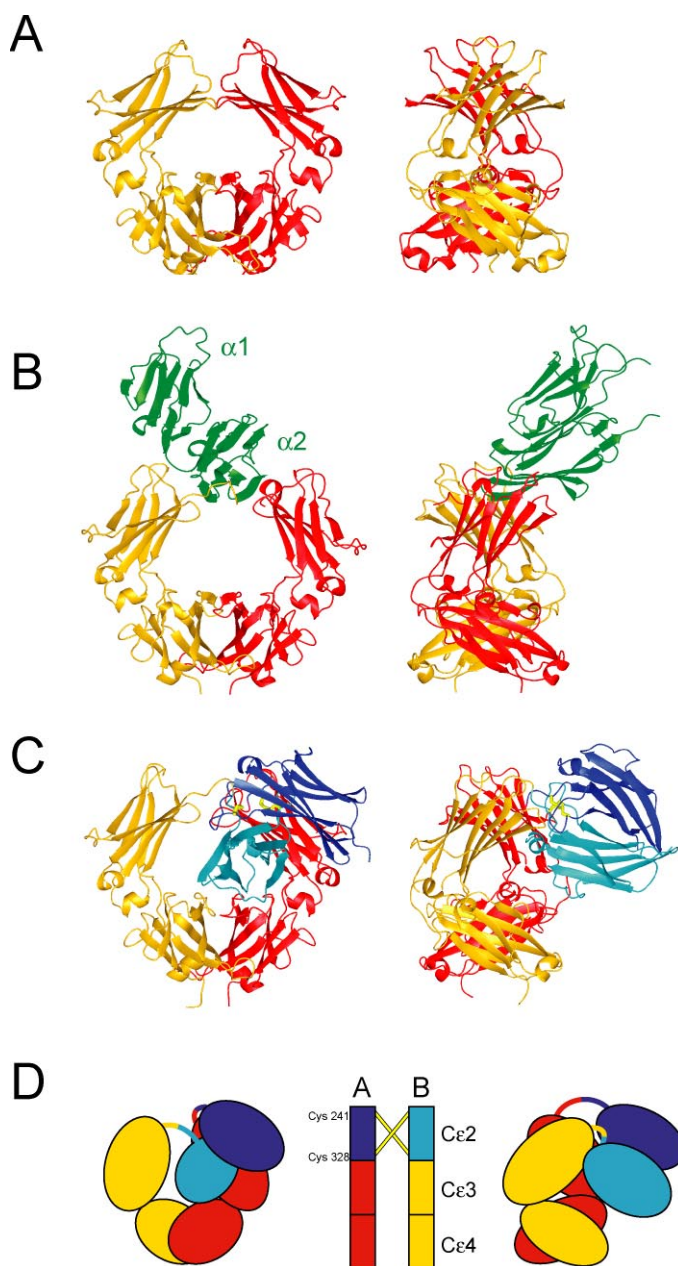


Figure 2 The structure of IgE and its interaction with Fc ϵ RI α . Ribbon representations of the polypeptide chain folds within the domains of (A) C ϵ 3–4 (31), (B) C ϵ 3–4 complexed with Fc ϵ RI α (37), and (C) Fc ϵ (45) in two orthogonal views, color-coded according to the schematic diagrams in (D).

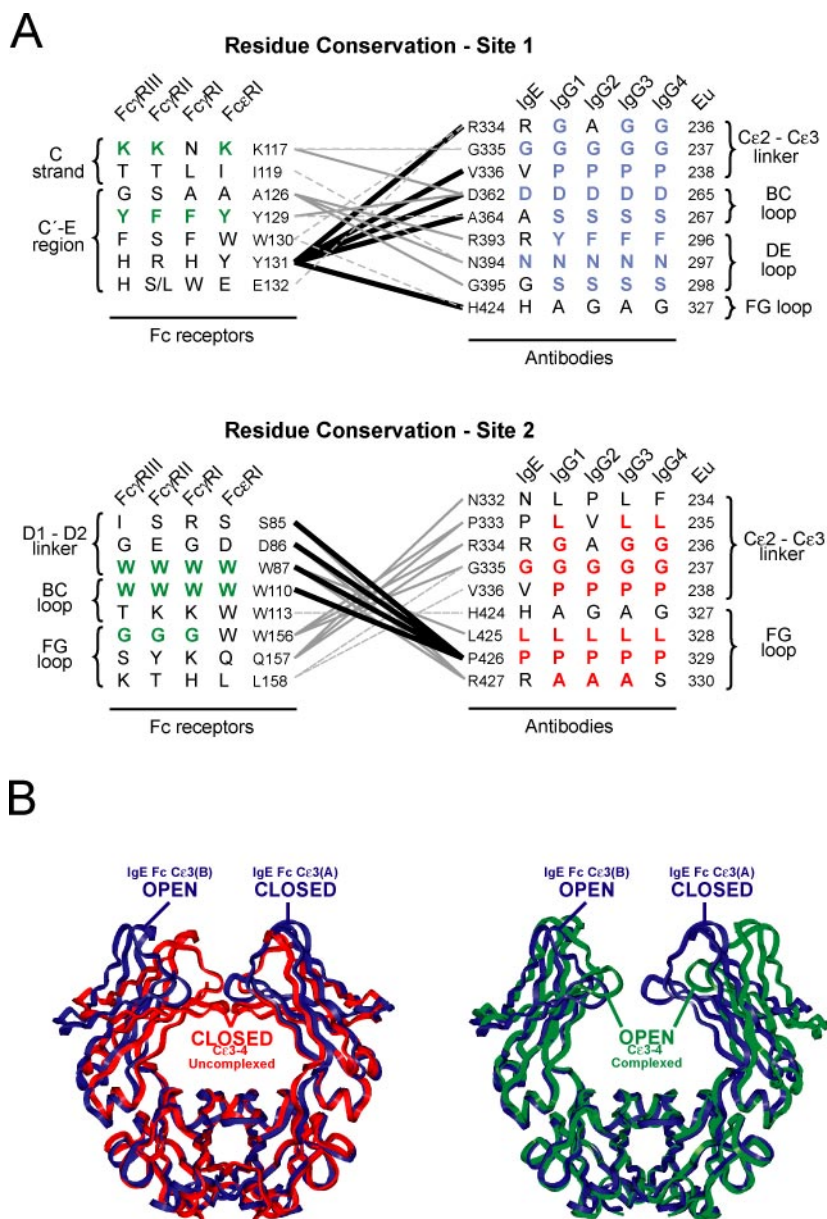


Figure 3 Structural details of the interaction between Fc ϵ and Fc ϵ RI α . (A) Conservation of amino acid residues and contacts of the C ϵ 3-4:Fc ϵ RI α interfaces in IgG and IgG receptors (adapted from Ref. 37). (B) Variation in quaternary structure of the C ϵ 3 and C ϵ 4 domains between Fc ϵ (blue) (45) and C ϵ 3-4 complexed (green) (37) and uncomplexed (red) (31), with the structures superimposed using their C ϵ 4 domains.

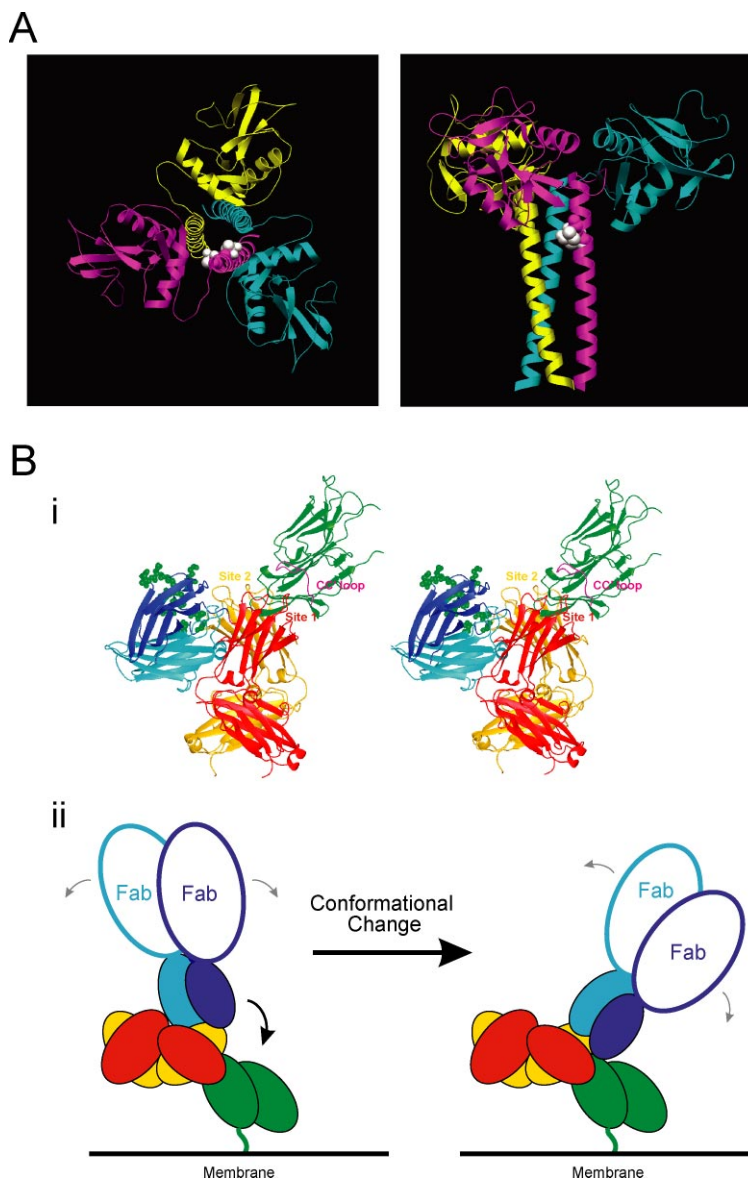


Figure 4 (A) Model of the structure of the CD23 lectin head showing the Der p1 cleavage site space filled in white (116a). (B) Modeling of the initial complex formed between IgE Fc and Fc ϵ RI α implies a conformational change on receptor binding. (i) Stereoscopic ribbon representation of Fc ϵ RI (green) docked onto Fc ϵ (colored as in Figure 2) (based on the contacts formed between C ϵ 3–4 and Fc ϵ RI α in Reference 37). The residues on C ϵ 2 identified by NMR to contact Fc ϵ RI α are shown green. Their location implies a substantial movement of C ϵ 2 upon receptor binding, shown schematically in (ii).

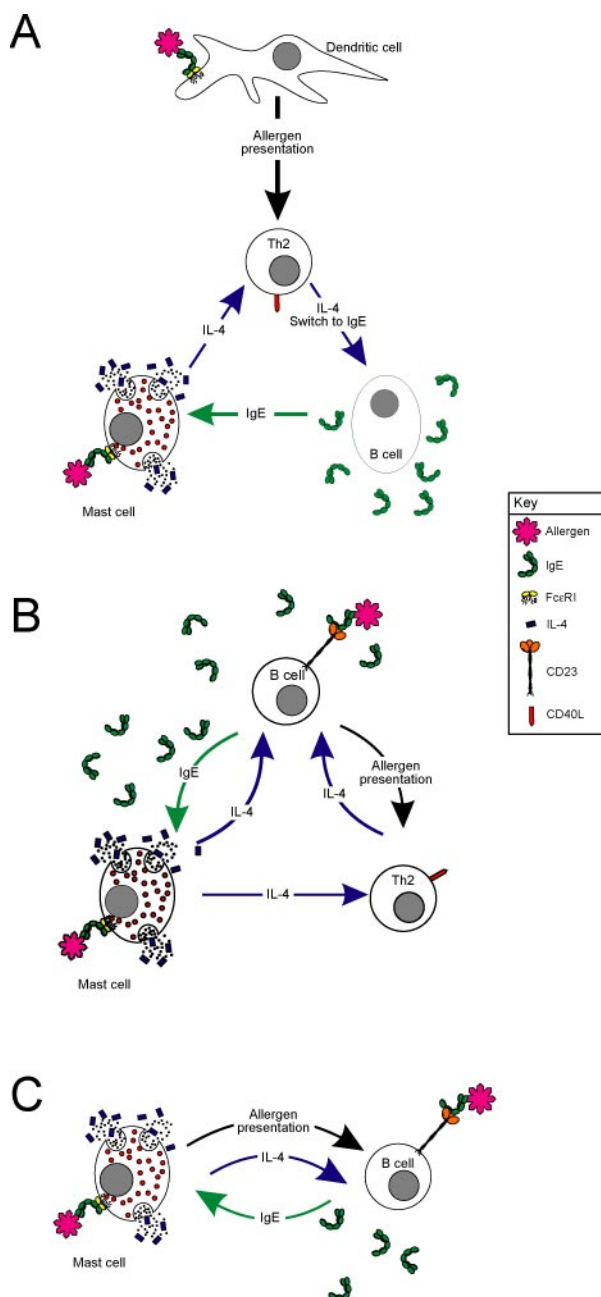


Figure 5 Positive feedback loops involving IgE synthesis, mast cell degranulation, and antigen presentation. The antigen-presenting cell may be a dendritic cell (A), a B cell (B) or a mast cell (C).

CONTENTS

FRONTISPIECE, <i>Thomas A. Waldmann</i>	x
THE MEANDERING 45-YEAR ODYSSEY OF A CLINICAL IMMUNOLOGIST, <i>Thomas A. Waldmann</i>	1
CD8 T CELL RESPONSES TO INFECTIOUS PATHOGENS, <i>Phillip Wong</i> <i>and Eric G. Pamer</i>	29
CONTROL OF APOPTOSIS IN THE IMMUNE SYSTEM: BCL-2, BH3-ONLY PROTEINS AND MORE, <i>Vanessa S. Marsden</i> <i>and Andreas Strasser</i>	71
CD45: A CRITICAL REGULATOR OF SIGNALING THRESHOLDS IN IMMUNE CELLS, <i>Michelle L. Hermiston, Zheng Xu, and Arthur Weiss</i>	107
POSITIVE AND NEGATIVE SELECTION OF T CELLS, <i>Timothy K. Starr,</i> <i>Stephen C. Jameson, and Kristin A. Hogquist</i>	139
IGA FC RECEPTORS, <i>Renato C. Monteiro and Jan G.J. van de Winkel</i>	177
REGULATORY MECHANISMS THAT DETERMINE THE DEVELOPMENT AND FUNCTION OF PLASMA CELLS, <i>Kathryn L. Calame, Kuo-I Lin,</i> <i>and Chainarong Tunyaplin</i>	205
BAFF AND APRIL: A TUTORIAL ON B CELL SURVIVAL, <i>Fabienne Mackay, Pascal Schneider, Paul Rennert, and Jeffrey Browning</i>	231
T CELL DYNAMICS IN HIV-1 INFECTION, <i>Daniel C. Douek,</i> <i>Louis J. Picker, and Richard A. Koup</i>	265
T CELL ANERGY, <i>Ronald H. Schwartz</i>	305
TOLL-LIKE RECEPTORS, <i>Kiyoshi Takeda, Tsuneyasu Kaisho,</i> <i>and Shizuo Akira</i>	335
POXVIRUSES AND IMMUNE EVASION, <i>Bruce T. Seet, J.B. Johnston,</i> <i>Craig R. Brunetti, John W. Barrett, Helen Everett, Cheryl Cameron,</i> <i>Joanna Sypula, Steven H. Nazarian, Alexandra Lucas,</i> <i>and Grant McFadden</i>	377
IL-13 EFFECTOR FUNCTIONS, <i>Thomas A. Wynn</i>	425
LOCATION IS EVERYTHING: LIPID RAFTS AND IMMUNE CELL SIGNALING, <i>Michelle Dykstra, Anu Cherukuri, Hae Won Sohn,</i> <i>Shiang-Jong Tzeng, and Susan K. Pierce</i>	457

THE REGULATORY ROLE OF V α 14 NKT CELLS IN INNATE AND ACQUIRED IMMUNE RESPONSE, <i>Masaru Taniguchi, Michishige Harada, Satoshi Kojo, Toshinori Nakayama, and Hiroshi Wakao</i>	483
ON NATURAL AND ARTIFICIAL VACCINATIONS, <i>Rolf M. Zinkernagel</i>	515
COLLECTINS AND FICOLINS: HUMORAL LECTINS OF THE INNATE IMMUNE DEFENSE, <i>Uffe Holmskov, Steffen Thiel, and Jens C. Jensenius</i>	547
THE BIOLOGY OF IGE AND THE BASIS OF ALLERGIC DISEASE, <i>Hannah J. Gould, Brian J. Sutton, Andrew J. Beavil, Rebecca L. Beavil, Natalie McCloskey, Heather A. Coker, David Fear, and Lyn Smurthwaite</i>	579
GENOMIC ORGANIZATION OF THE MAMMALIAN MHC, <i>Attila Kumánovics, Toyoyuki Takada, and Kirsten Fischer Lindahl</i>	629
MOLECULAR INTERACTIONS MEDIATING T CELL ANTIGEN RECOGNITION, <i>P. Anton van der Merwe and Simon J. Davis</i>	659
TOLEROGENIC DENDRITIC CELLS, <i>Ralph M. Steinman, Daniel Hawiger, and Michel C. Nussenzweig</i>	685
MOLECULAR MECHANISMS REGULATING TH1 IMMUNE RESPONSES, <i>Susanne J. Szabo, Brandon M. Sullivan, Stanford L. Peng, and Laurie H. Glimcher</i>	713
BIOLOGY OF HEMATOPOIETIC STEM CELLS AND PROGENITORS: IMPLICATIONS FOR CLINICAL APPLICATION, <i>Motonari Kondo, Amy J. Wagers, Markus G. Manz, Susan S. Prohaska, David C. Scherer, Georg F. Beilhack, Judith A. Shizuru, and Irving L. Weissman</i>	759
DOES THE IMMUNE SYSTEM SEE TUMORS AS FOREIGN OR SELF? <i>Drew Pardoll</i>	807
B CELL CHRONIC LYMPHOCYTIC LEUKEMIA: LESSONS LEARNED FROM STUDIES OF THE B CELL ANTIGEN RECEPTOR, <i>Nicholas Chiorazzi and Manlio Ferrarini</i>	841
INDEXES	
Subject Index	895
Cumulative Index of Contributing Authors, Volumes 11–21	925
Cumulative Index of Chapter Titles, Volumes 11–21	932

ERRATA

An online log of corrections to *Annual Review of Immunology* chapters may be found at <http://immunol.annualreviews.org/errata.shtml>