

Annual Review of Immunology

IgE, IgE Receptors and Anti-IgE Biologics: Protein Structures and Mechanisms of Action

J.M. McDonnell,¹ B. Dhaliwal,² B.J. Sutton,¹
and H.J. Gould¹

¹Randall Centre for Cell and Molecular Biophysics and School of Basic and Medical Biosciences, King's College London, London, UK; email: james.mcdonnell@kcl.ac.uk, brian.sutton@kcl.ac.uk, hannah.gould@kcl.ac.uk

²Almirall, Barcelona, Spain; email: balvinder.dhaliwal@almirall.com

Annu. Rev. Immunol. 2023. 41:255–75

First published as a Review in Advance on
February 3, 2023

The *Annual Review of Immunology* is online at
immunol.annualreviews.org

<https://doi.org/10.1146/annurev-immunol-061020-053712>

Copyright © 2023 by the author(s). This work is licensed under a Creative Commons Attribution 4.0 International License, which permits unrestricted use, distribution, and reproduction in any medium, provided the original author and source are credited. See credit lines of images or other third-party material in this article for license information.

**ANNUAL
REVIEWS CONNECT**

www.annualreviews.org

- Download figures
- Navigate cited references
- Keyword search
- Explore related articles
- Share via email or social media

Keywords

immunoglobulins, Fc receptors, immunoglobulin E, FcεRI, CD23, allergy, allostery

Abstract

The evolution of IgE in mammals added an extra layer of immune protection at body surfaces to provide a rapid and local response against antigens from the environment. The IgE immune response employs potent expulsive and inflammatory forces against local antigen provocation, at the risk of damaging host tissues and causing allergic disease. Two well-known IgE receptors, the high-affinity FcεRI and low-affinity CD23, mediate the activities of IgE. Unlike other known antibody receptors, CD23 also regulates IgE expression, maintaining IgE homeostasis. This mechanism evolved by adapting the function of the complement receptor CD21. Recent insights into the dynamic character of IgE structure, its resultant capacity for allosteric modulation, and the potential for ligand-induced dissociation have revealed previously unappreciated mechanisms for regulation of IgE and IgE complexes. We describe recent research, highlighting structural studies of the IgE network of proteins to analyze the uniquely versatile activities of IgE and anti-IgE biologics.

INTRODUCTION

IgE is best known for its role in allergic responses, but it is thought to have evolved for protection against parasites and toxins through powerful inflammatory and expulsive reactions that in sensitized individuals are elicited immediately on contact with antigen (1). FcεRI, the high-affinity IgE receptor found mainly on mast cells and basophils, is primarily responsible for this allergic sensitization, in which minute amounts of allergen cross-link FcεRI-bound IgE, triggering cell degranulation and releasing proinflammatory mediators. CD23, the low-affinity IgE receptor (also known as FcεRII), found mainly on B cells, has a plethora of functions, including regulation of IgE synthesis. This review focuses on the results of structural and biophysical studies of human IgE and its interactions with IgE receptors (**Figure 1a–c**) that have provided a deeper understanding of effector functions of IgE in immediate hypersensitivity and IgE homeostasis.

The general characteristics of IgE and IgE–receptor and IgE–anti-IgE protein complexes have been described in previous reviews (1–3). Notably, IgE, like IgM, has four constant heavy (C_H) domains, with C_{H2} ($C_{\epsilon 2}$ in IgE) domains replacing the hinge region present in the other switched antibody classes (IgD, IgG, IgA). A second crucial feature is its unique flexibility in the Fc region, including the asymmetric bending of the $C_{\epsilon 2}$ domains (4), a large variation in the $C_{\epsilon 3}$ – $C_{\epsilon 4}$ angle resulting in open and closed conformations of IgE–Fc (4), and an unusual amount of intradomain flexibility in the $C_{\epsilon 3}$ domains (5, 6). These attributes are responsible for the extremely high-affinity interaction with the FcεRI receptor and long persistence of allergic sensitivity compared with immune reactions caused by other antibody isotypes. IgE homeostasis, again a unique feature of this isotype, is provided by the actions of CD23 with CD21 as a coreceptor. This intricate system of regulation is in large part due to the inherent flexibility of the IgE, as well as FcεRI and CD23 molecules that enable IgE to function independently through its two different receptors. An understanding of these properties has been leveraged in the development of anti-IgE biologics.

To determine the three-dimensional structures of IgE and its receptors and characterize their critical interactions using protein crystallography and nuclear magnetic resonance (NMR) spectroscopy, it has been necessary to generate soluble recombinant fragments corresponding to smaller functional domains that retain full binding activity. These fragments are illustrated in **Figure 1**, with schematic diagrams showing the complete molecules for context.

FcεRI BINDS IgE WITH HIGH AFFINITY

FcεRI is expressed on mast cells and basophils, the chief effector cells for IgE (**Figure 1a**), as a tetramer of an α chain containing the extracellular IgE-binding sites, with a β chain and two γ chains containing intracellular immunoreceptor tyrosine-activation motifs (ITAMs) (**Figure 1b**) that activate the cellular response on antigen binding (7). FcεRI is also present on other leukocytes, airway smooth muscle cells, and mucosal epithelial cells either as an $\alpha\beta\gamma_2$ tetramer or as an $\alpha\gamma_2$ trimer. Mast cells and basophils express FcεRI at extremely high levels, approximately 100,000 (10^5) molecules per cell, and because of the high affinity ($K_D \approx 10^{-10}$ M) of the FcεRI–antibody interaction, the receptors are almost always occupied by IgE in vivo, despite the low physiological concentrations of IgE in circulation. For this reason, minute amounts of multivalent antigen (allergen) can cross-link the FcεRI–IgE complexes and initiate rapid cell activation, leading to immediate cell degranulation with the release of histamine, inflammatory mediators, and vasodilators and consequent allergic and inflammatory responses (7).

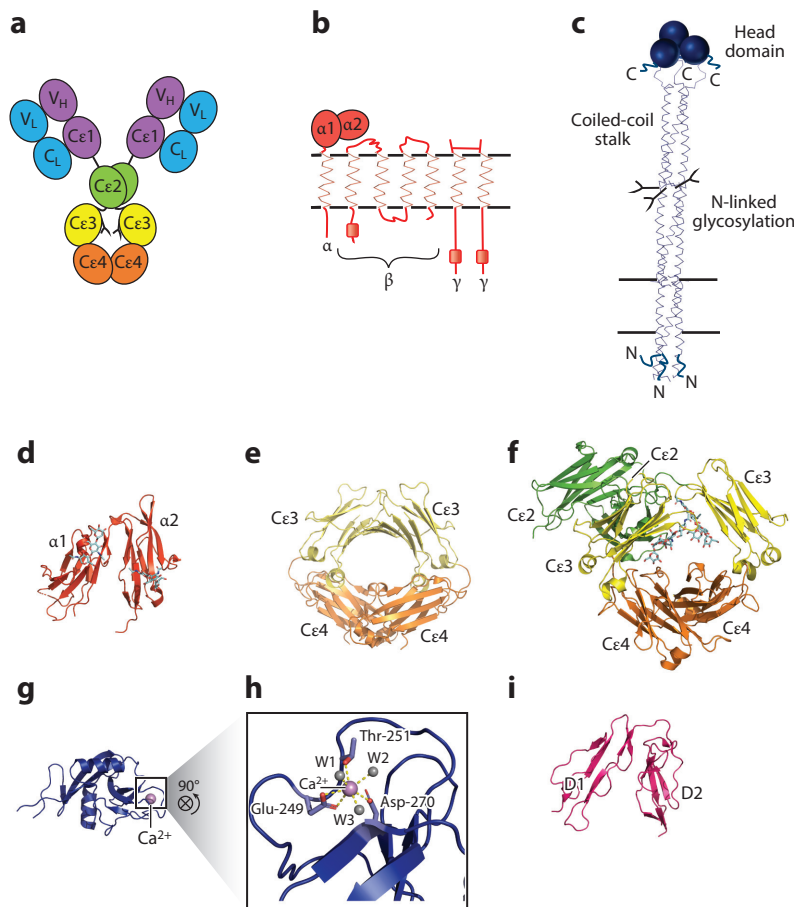


Figure 1

Proteins of the IgE network. (*a–c*) Schematic diagrams of IgE and its receptors. (*a*) The domain structure of the IgE antibody. Only the conserved glycosylation site of Cε3, found in all mammalian antibody isotypes, is depicted. The Cε2 domains are linked by disulfide bonds (not shown). (*b*) The four chains of FcεRI. The two γ chains are linked by a disulfide. The ITAMs of the β and γ chains (red rectangles) initiate the signaling pathway. The glycosylation sites of domains α1 and α2 are not shown. (*c*) The membrane-bound CD23 homotrimer. (*d–i*) A representative selection of protein fragments used in structural and biophysical studies. (*d*) sFcεRIα (PDB ID 1F2Q). (*e*) Fcε3–4 (PDB ID 1FP5). (*f*) IgE-Fc (PDB ID 2WQR), with the same color scheme as in panel *a*; carbohydrate structures are represented as sticks here. (*g*) The structure of the naturally occurring CD23 fragment derCD23 (PDB ID 4G9A). (*h*) Enlarged view showing Ca²⁺ binding to the principal binding site: Glu-249, Thr-251, and Asp-270 provide four oxygen atoms, and three water molecules (labeled W1, W2, and W3) provide three oxygen atoms (yellow dashed lines). (*i*) CD21 D1-D2 (PDB ID 1LY2). Abbreviations: ITAM, immunoreceptor tyrosine-activation motif; PDB ID, Protein Data Bank identifier.

STRUCTURES OF sFcεRIα AND ITS COMPLEX WITH sFcεRIα AND Fcε3–4 ENABLED A PRELIMINARY UNDERSTANDING OF THE IgE-FcεRI INTERACTION

The first functional fragment to provide structural insights into IgE binding by its high-affinity receptor was a soluble moiety of the extracellular region of the FcεRI α chain (sFcεRIα) (8). This

construct contains two immunoglobulin-like domains, $\alpha 1$ and $\alpha 2$, separated by a short, flexible linker sequence. The $\alpha 1$ domain is bent back over $\alpha 2$ to form an arch with the IgE-binding site at the top (**Figure 1d**). It is a hydrophobic region where a prominent loop in one of the β strands is near a tryptophan-rich region that mutagenesis implicated in IgE binding (9). The sequences of $\alpha 1$ and $\alpha 2$ are homologous, but $\alpha 2$ differs from $\alpha 1$ in having two bulky tyrosine residues in the hydrophobic core. This results in separation of two β strands at the surface of $\alpha 2$, exposing two subsites for IgE binding in $\alpha 2$ and the linker to $\alpha 1$. The extracellular Fc ϵ RI α domains are heavily glycosylated, but the glycosylation sites surround the IgE-binding site, leaving it exposed, and are not involved in IgE binding.

Despite the twofold symmetry of the ϵ -heavy chain dimer, IgE-Fc binds to Fc ϵ RI α with 1:1 stoichiometry (10). The crystal structure of sFc ϵ RI α bound to a subfragment of IgE-Fc consisting of the dimer of C ϵ 3 and C ϵ 4 domains (Fc ϵ 3–4) (11) showed that the key to the 1:1 stoichiometry of the Fc ϵ RI α -IgE complex is the presence of two nonidentical subsites. One is present on each of the two C ϵ 3 domains in IgE, and they bind to a single Fc ϵ RI α molecule. Subsite 1 involves contact between one C ϵ 3 domain (C ϵ 3A) and the C-C' loop region of the receptor $\alpha 2$ domain, while subsite 2 involves interactions between the other C ϵ 3 domain (C ϵ 3B) and the linker region between $\alpha 1$ and $\alpha 2$, and the B-C and F-G loops of $\alpha 2$ (**Figure 2a**). Proline 426 from the C ϵ 3B domain intercalates between sFc ϵ RI α residues tryptophan 87 and tryptophan 110, forming a hydrophobic proline sandwich that is also a conserved feature of IgG antibodies and their complexes with the Fc γ R receptor family (12).

Seven of the 15 sFc ϵ RI α amino acids at the interface with Fc ϵ 3–4 are bulky aromatic residues, of which 5 are exposed tryptophans. The relatively large number of aromatic receptor residues at the

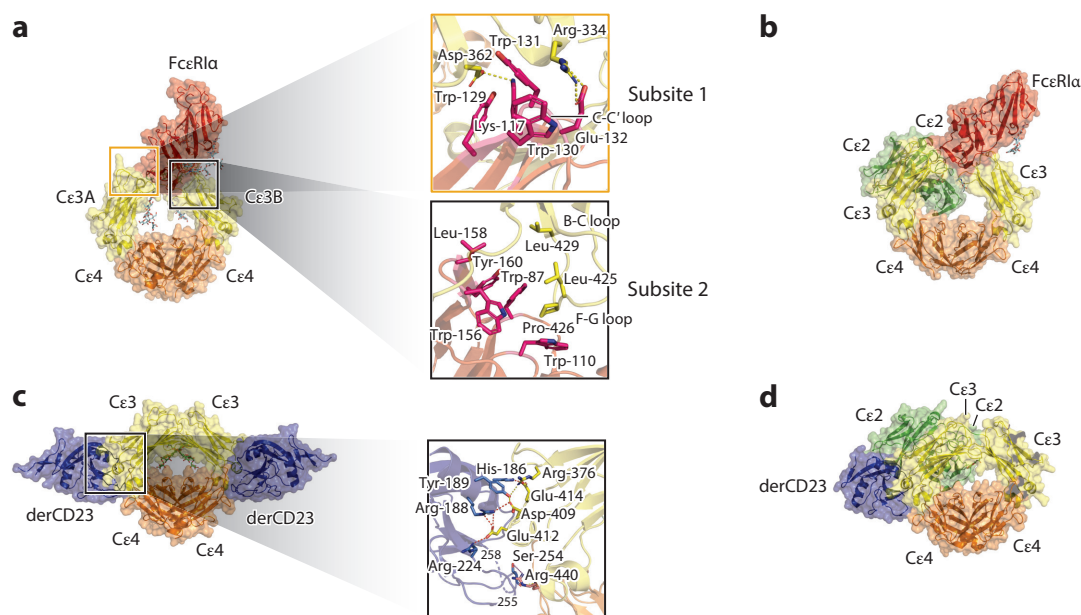


Figure 2

IgE-receptor interactions. (a) The Fc ϵ 3–4/sFc ϵ RI α complex (PDB ID 1F6A). Insets show enlarged views of the interface at subsites 1 and 2. (b) IgE-Fc/sFc ϵ RI α (PDB ID 2Y7Q). (c) Fc ϵ 3–4/derCD23 (PDB ID 4EZM). Inset is an enlarged view of the interface, with the four salt bridges shown in red, additional hydrogen bonds present in all six independent interactions shown in yellow, and a further hydrogen bond present in five of six molecules shown in purple. (d) IgE-Fc/derCD23 (PDB ID 5LGK). Abbreviation: PDB ID, Protein Data Bank identifier.

interface, together with the large buried surface area in the complex, helps account for the greater affinity of this complex ($K_D \approx 10^{-10}$ M) compared with homologous Fc γ R-IgG interactions (K_D values typically in the range of 10^{-6} to 10^{-8} M for the family of Fc γ receptors).

Additionally, the C' strand of Fc ϵ RI α , part of the C-C' loop region at the interface with Fc ϵ 3–4, is distorted on binding, so that the C' strand in domain $\alpha 2$ is longer in the complex, enabling additional hydrogen bonding to the C strand compared with homologous structures in IgG receptors.

The Fc ϵ 3–4/sFc ϵ RI α complex revealed the structure of the Fc ϵ 3–4 region of IgE and its interactions with sFc ϵ RI α , but the part played by the interdomain motions of Fc ϵ 3–4, the intradomain flexibility of the C ϵ 3 domains, and crucial allosteric interactions mediated by the C ϵ 2 domains became clear only from the structures of the unbound Fc ϵ 3–4 domains and of the complete Fc region including C ϵ 2, C ϵ 3 and C ϵ 4, both free and when bound to sFc ϵ RI α (4, 13, 14).

C ϵ 3 IS HIGHLY DYNAMIC

The publication of unbound Fc ϵ 3–4 crystal structures (13, 15, 16) (**Figure 1e**) showed that this homodimer can adopt a range of conformations. The dimeric moiety showed a high degree of flexibility; taking an axis approximately perpendicular to the C ϵ 3–C ϵ 4 dimer axis, an approximately 13° rotational difference between the two C ϵ 3 domains of the free C ϵ 3–C ϵ 4 dimer was observed. Further analyses revealed an extremely open conformation, with separation of the upper portions of the C ϵ 3 domains that allows binding to sFc ϵ RI α , to a closed conformation, predominant in the free Fc ϵ 3–4, in which the C ϵ 3 domains are closer to one another, enabling binding to CD23 (see the section titled *derCD23 Binds Asymmetrically to IgE-Fc*). The C ϵ 3–C ϵ 4 interdomain angle is seen to vary over a range of $\sim 25^\circ$ (16).

In addition to the open and closed orientations of C ϵ 3 relative to the C ϵ 4 domain, the C ϵ 3 domain is inherently flexible: As a monomer in solution it is in a largely unstructured, molten globule-like state (5, 6). This has been attributed to a poorly packed hydrophobic core, an unusually high electrostatic charge, and an unusually low content of hydrophobic residues (17), contributing to IgE's susceptibility to thermal unfolding and low melting temperature compared with IgG (16), where the homologous domain (C γ 2) has the structure and dynamics of a more typical immunoglobulin domain (18).

Regions of inherent disorder allow modulation of specificity and affinity of protein interactions (19), and on interaction with sFc ϵ RI α , C ϵ 3 adopts a well-defined tertiary structure (20), as predicted from studies on the isolated domain (17).

THE PRESENCE OF THE C ϵ 2 DOMAIN PAIR AND THE INHERENT FLEXIBILITY OF C ϵ 3 EXPLAIN THE SLOW DISSOCIATION RATE OF IgE FROM Fc ϵ RI

The critical part played by C ϵ 2 in the high-affinity interaction of IgE with Fc ϵ RI first emerged from studies comparing the kinetics of binding of sFc ϵ RI α to the full-length IgE, to IgE-Fc, and to Fc ϵ 3–4 (21). These studies showed that while the absence of the Fab domains made little difference to the kinetics of the interaction of the constant domains with sFc ϵ RI α (IgE/sFc ϵ RI: $k_{on} = 2.4 \times 10^5$ M $^{-1}$ s $^{-1}$, $k_{off} = 1.8 \times 10^{-4}$ s $^{-1}$, $K_D = 7.5 \times 10^{-10}$ M; IgE-Fc/sFc ϵ RI α : $k_{on} = 3.0 \times 10^5$ M $^{-1}$ s $^{-1}$, $k_{off} = 1.9 \times 10^{-4}$ s $^{-1}$, $K_D = 6.3 \times 10^{-10}$ M), deletion of the C ϵ 2 domains from the IgE Fc fragment, to leave only Fc ϵ 3–4, increases the rate of dissociation from sFc ϵ RI α more than tenfold (Fc ϵ 3–4/sFc ϵ RI α : $k_{on} = 9.2 \times 10^5$ M $^{-1}$ s $^{-1}$, $k_{off} = 3.4 \times 10^{-3}$ s $^{-1}$, $K_D = 3.7 \times 10^{-9}$ M) (21).

The structural basis for this dramatic effect became clear only on elucidation of the crystal structure of the complete IgE-Fc bound to sFc ϵ RI α (14) (**Figure 2b**). It was already known from

earlier studies (4, 22, 23) that free IgE-Fc adopts a bent conformation in which the Cε2 of one ε chain, designated chain B, is asymmetrically bent over the other ε chain, chain A, contacting both Cε3 and Cε4 in chain A (**Figure 1f**). The structure of the IgE-Fc/sFcεRIα complex showed that when bound to sFcεRIα, the IgE-Fc is more acutely bent and compact than when unbound, but although the Cε2 domains are shifted with respect to the Cε3 domains, the Cε2 is not brought into direct contact with the receptor; the influence of the Cε2 domains on receptor binding therefore operates indirectly through contact with the Cε3 domains, and the resulting changes in dynamics and conformation.

One striking thermodynamic difference between the IgE-Fc/sFcεRIα and Fcε3–4/sFcεRIα interactions is that the Fcε3–4/sFcεRIα interaction is enthalpically driven ($\Delta H = -37.0 \text{ kJ mol}^{-1}$) with a small favorable entropic component ($T\Delta S = +11.0 \text{ kJ mol}^{-1}$), whereas the IgE-Fc/sFcεRIα interaction is entropically driven ($T\Delta S = +51.0 \text{ kJ mol}^{-1}$), with a negligible unfavorable enthalpic component ($\Delta H = +0.3 \text{ kJ mol}^{-1}$) (14).

To summarize, the Cε2 domain pair contributes to greater order of the intrinsically flexible Cε3 domains prior to FcεRI binding, thereby minimizing the loss of conformational entropy upon complex formation. This results in greater stability of the complex with FcεRIα, manifested by markedly slower dissociation rates (14).

Recently, low-resolution cryo-electron microscopy (cryo-EM) structures of intact IgE have been elucidated for the first time (24). The structures, together with small-angle X-ray scattering studies, show that the IgE-Fc moiety in IgE can be significantly less bent than that in the isolated IgE-Fc fragment, but this is unlikely to change the considerations above substantially, given the similarity of the binding kinetics in the interactions of the complete IgE and IgE-Fc with sFcεRIα (21).

CD23 IS A MULTIFUNCTIONAL C-TYPE LECTIN IMPLICATED IN IgE HOMEOSTASIS

Unlike IgE and FcεRI, along with the FcγRs, which are all members of the Ig superfamily, the second receptor for IgE (FcεRII), now better known as CD23, is a type II membrane protein belonging to the C-type lectin domain (CTLD) superfamily (25, 26). Some CTLD-containing proteins interact with the carbohydrate moieties of glycoproteins via a bound calcium ion (Ca^{2+}), while others bind neither carbohydrate nor Ca^{2+} and interact with other ligands, including proteins (27). In its cell membrane-bound form, CD23 consists of three CTLD head domains connected to the membrane by a trimeric α-helical coiled-coil stalk region (28) (**Figure 1c**), which projects the CTLD head domains away from the cell membrane, facilitating surveillance for IgE. CD23 is a multifunctional protein (1) with two alternative C-terminal cytoplasmic sequences: CD23a, which is expressed by B cells that participate in antigen presentation, and CD23b, which is expressed by various leukocytes that effect cellular cytotoxicity (3). Here we are concerned with its activity in IgE homeostasis in B cells (29). CD23 has been implicated both in the activation of IgE production and in its downregulation (30), whereby it is produced at much lower abundance than other antibody isotypes: 2 ng/mL for IgE compared with 10 mg/mL of IgG in serum. Hibbert et al. (31) have proposed an autoregulatory mechanism involving complex formation on the B cell surface by different combinations of IgE with CD23 and CD21 and that is supported by compelling experimental evidence, at least in vitro (29).

The lectin head domain is the minimal structure required for full activity in binding to IgE. CD23 monomers bind to CD23 with relatively low affinity, compared with FcεRI. However, as CD23 is expressed on the cell membrane, avidity can substantially enhance the interaction with the homodimeric IgE molecule (30). The stalk region of CD23 is susceptible to attack by

endogenous proteases, most notably ADAM10 (32), releasing soluble trimeric and monomeric forms of CD23. These can be further degraded to smaller fragments by exogenous proteases. For example, a protease from a house dust mite allergen, Der p I, cleaves off the stalk and tail from the CD23 receptor, leaving a soluble head domain that has been replicated as a recombinant fragment, derCD23 (**Figure 1g**), commonly used in structural studies of CD23 and its complexes with IgE-Fc and various subfragments of IgE-Fc.

In the autoregulation of IgE production *in vivo*, opposing roles have been proposed for the membrane and soluble forms of CD23, interacting with membrane IgE and CD21, and with secreted and membrane IgE, respectively, to provide IgE homeostasis (**Supplemental Figure 1**). We describe what is known about the interactions of these molecules and how they contribute to this model of IgE homeostasis.

Supplemental Material >

THE HEAD DOMAIN OF CD23 IS A CANONICAL LECTIN DOMAIN BUT DOES NOT REQUIRE CARBOHYDRATE OR CALCIUM TO BIND TO HUMAN IgE

The lectin head domain of CD23 has the same canonical structure as other members of the lectin superfamily. Lectin proteins are best known for their carbohydrate recognition domain (CRD), but as mentioned above, many lectins bind only to protein (27, 33). The binding of calcium ions to two main canonical sites, the principal Ca^{2+} -binding site and the auxiliary Ca^{2+} site, in the lectin framework is required for C-type lectin binding to carbohydrates. Human IgE-Fc has three N-glycosylation sites in two specific exposed locations on the surface of C ϵ 2 and C ϵ 3, and one site in C ϵ 3, conserved with IgG and other isotypes, in the interior of IgE-Fc (**Figure 1f**). Thus, the CD23 lectin domain was seen as a potential CRD. Upon investigation, however, it was found that calcium, but not carbohydrate, is required for mouse CD23 binding to IgE (34). Then, surprisingly, it was found that human CD23 could bind to an aglycosylated fragment of human IgE (35). The conflicting results from human and mouse excited the question of whether or not the lectin domain of CD23 is indeed a calcium-dependent CRD. The conundrum inspired two separate lines of investigation, one to find an alternative ligand for human CD23, and the other to examine the structure of the binding sites for calcium and carbohydrate and their involvement in IgE recognition and affinity.

On the hypothesis that human CD23 binding to IgE is carbohydrate independent, one investigation sought to discover an alternative ligand for CD23 and led to the discovery of CD21 as a binding partner (36). CD21 was already well known as complement receptor 2 (CR2), binding to proteins of the complement system (for example the C3d fragment) (37), gp350/220 envelope protein of Epstein-Barr virus (38), and IFN- α (39), but it had not been implicated in CD23 actions.

The role of calcium in IgE binding by CD23 emerged from the structural studies described below.

CALCIUM BINDING TO derCD23 STABILIZES THE CONFORMATION OF FLEXIBLE LOOPS AT THE IgE-BINDING SITE

NMR (31) and X-ray crystallography (40) studies show that the derCD23 domain consists of two roughly orthogonal α helices and eight β strands forming two antiparallel β sheets (**Figure 1g**). Four disulfide bonds contribute to the tertiary structure, with a hydrophobic core formed by seven tryptophan residues and other conserved side chains, and two flexible loops, loop 1 and loop 4, are present at the IgE-binding site (41). The trimeric model for full-length CD23 (28), as described above, is consistent with the findings that the crystal structures of other members of the C-type lectin family are homotrimeric (42).

The carbohydrates galactose, glucose, lactose, mannose, and *N*-acetylglucosamine do not bind to derCD23 even at the highest concentrations of 125 mM (31), but a Ca^{2+} ion binds at the principal binding site (33, 40) ($K_D \approx 1.8 \times 10^{-3}$ M) (31) (**Figure 1b**).

Comparison of the tertiary structures of the Ca^{2+} -free and -bound derCD23 shows little variation, apart from considerable conformational plasticity in loop 1 and loop 4, known to contribute to IgE binding (41). Whereas in the absence of Ca^{2+} , loop 4 shows considerable conformational variation, loop 4 conformations become largely ordered in the presence of Ca^{2+} . The isomerization state of proline 250, part of the canonical motif of EPN/T or the QPD motif found in C-type lectins (25), appears to be a key determinant of the conformation of loop 4. Proline 250 is found in either a *cis* or *trans* configuration in the Ca^{2+} -free structures, but it is locked in *cis* in Ca^{2+} -bound derCD23 (43, 44).

An analysis of six additional crystal forms of derCD23 (44) confirmed these findings and revealed that the movements of the IgE-binding loops 1 and 4 are coupled. The modulation of the conformation of loop 4, and thus loop 1, suggests a mechanism by which Ca^{2+} binding to derCD23 enhances its affinity for IgE-Fc (discussed in the section titled Calcium Modulates the IgE/CD23 Interaction).

CD23 BINDS TO CLOSED CONFORMATIONS OF Fcε3–4 THAT PRECLUDE BINDING BY FcεRI

The CD23 binding site maps mainly to the Cε3 domain of IgE (45, 46), and monomeric CD23 binds to Fcε3–4 and IgE-Fc with 2:1 stoichiometry (30, 45–47) (**Figure 2c**). Although IgE can bind two molecules of CD23, when the complex is formed with the CD23 trimer, the two head domains cannot come from the same trimer. This is because the diametrically opposed head domains are 136 Å apart and therefore, if from the same trimer, would require a third of the α -helical coiled-coil stalk to unravel. Instead, two head domains belonging to two different trimeric CD23 molecules could be cross-linked by a single IgE molecule, or a single trimeric CD23 could cross-link two to three IgEs (45). Further studies in which CD23 was bound to Fcε3–4 or IgE-Fc showed substantial asymmetry in the affinity of CD23 binding to the two chains (48). This is discussed further below.

Although both the CD23-binding site and FcεRI-binding site on IgE principally involve the Cε3 domain, they are distant from one another, as they are located at opposite ends of the Cε3 domain, and the CD23-binding site does not overlap with the FcεRI-binding site. This showed that the mutual inhibition of FcεRI and CD23 binding seen in previous studies (49) could not be due to orthosteric competition. The nonoverlapping receptor binding sites are indicative of an allosteric inhibitory mechanism. Superposition of the Fcε3–4/sFcεRIα and Fcε3–4/derCD23 complexes and unliganded Fcε3–4 structures on their Cε4 domain pairs shows that sFcεRIα binds to an extreme open conformation of Fcε3–4, while derCD23 binds to an extreme closed conformation of Fcε3–4. In the open, sFcεRIα-bound structure, steric clashes would occur to prevent derCD23 binding to Fcε3–4, while in the closed, derCD23-bound structure, steric clashes would occur with sFcεRIα, together revealing a mechanism of reciprocal allosteric inhibition (**Figure 3**). The mutual inhibition of sFcεRI and derCD23 binding to Fcε3–4 or IgE-Fc was verified in solution using competitive binding FRET and functional cellular assays (45, 46).

The mutually exclusive binding of IgE to its two receptors is biologically critical, as it prevents cross-linking of FcεRI-bound IgE on mast cells and basophils by trimeric sCD23 (31). Similarly, it prevents membrane CD23-IgE complexes on B cells or antigen-presenting cells or soluble IgE-CD23 complexes cross-linking FcεRI, eliminating the possibility of triggering an allergic reaction in the absence of allergen and potentially lethal anaphylaxis.

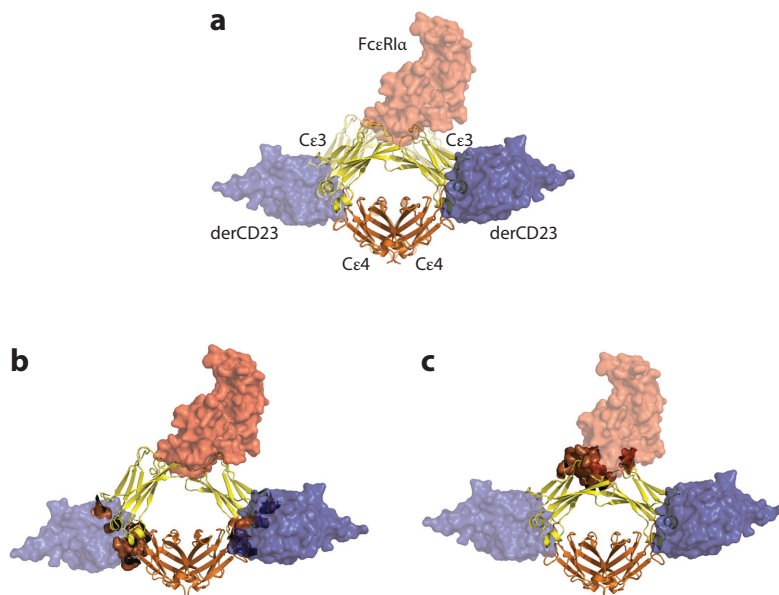


Figure 3

Superposition of the derCD23 and sFcεRIα complexes with Fcε3-4 to show the reciprocal allosteric inhibition of receptor binding. (a) Fcε3-4/derCD23 and Fcε3-4/sFcεRIα complexes superposed on the Cε4 domain pairs. Fcε3-4 bound to derCD23 adopts a closed conformation, whereas Fcε3-4 (*faded area*) bound to sFcεRIα is in an open conformation. (b) Steric clashes between the sFcεRIα structure and Fcε3-4/derCD23 complex are shown (*brown surfaces*). (c) Steric clashes between both derCD23 structures with the Fcε3-4/sFcεRIα complex are shown (*brown surfaces*).

CALCIUM MODULATES THE IgE/CD23 INTERACTION

Thermodynamic analysis of the IgE-Fc/derCD23 interaction showed that at 35°C it has a K_D of 2.0×10^{-6} M in the presence and 5.8×10^{-5} M in the absence of Ca^{2+} , making approximately a 30-fold enhancement of affinity in the presence of calcium (43). Moreover, it was established that in the Ca^{2+} -bound state, the IgE-Fc/derCD23 interaction is largely entropically driven. In contrast, in the Ca^{2+} -free state the interaction is driven by favorable enthalpy (discussed below).

The Ca^{2+} -bound Fcε3-4/derCD23 complex crystal structure (43) showed that two additional salt bridges are formed in the Ca^{2+} -bound structure, as well as two new hydrogen bonds, although two that were present in the Ca^{2+} -free structure are lost. These additional salt bridges, Asp227 (from derCD23 loop 1) to Arg440 and Asp258 (from derCD23 loop 4) to Arg440, make the principal contribution to the enhanced affinity of CD23 for IgE in the presence of Ca^{2+} .

Comparative analysis of the Ca^{2+} -bound and Ca^{2+} -free crystal structures of derCD23 and Fcε3-4/derCD23 complexes showed how Ca^{2+} binding to CD23 enhances the affinity for IgE: a Ca^{2+} ion binds at the principal binding site of the head domain of CD23, inducing Pro250 of mobile loop 4 to adopt a *cis* configuration, as described earlier, with concomitant conformational changes in the rest of the loop, propagating the coupled movement of loop 1. All of these changes upon Ca^{2+} binding give rise to a more entropically favorable IgE/CD23 interaction and an increase in affinity. This favorable entropic contribution is likely due to the ordering of the inherently flexible loops 1 and 4 of derCD23, resulting in a reduced entropic penalty upon binding IgE-Fc (43).

The crystal structures of murine and bovine derCD23 were recently determined. In contrast to human derCD23, bovine and murine derCD23 molecules bind Ca^{2+} at both the principal and auxiliary binding sites (50, 51). Interestingly, in human derCD23 residue Asp258 forms part of the defunct auxiliary binding site. It appears that by co-opting this residue, the human receptor lost a second Ca^{2+} binding site, but gained enhanced binding to IgE.

The effect of calcium on the affinity of CD23 for IgE is also likely to be important in the facilitation of antigen presentation by CD23. This requires internalization of a bound IgE-allergen complex “cargo” by endocytosis, and the release of the bound cargo for lysosomal degradation and processing, with the generation of peptides for binding and presentation by MHC molecules, and recycling of CD23 to the cell surface (52). Ca^{2+} binds to derCD23 with a K_D of about 1.5×10^{-3} M, approximately the same order of magnitude as the extracellular concentration of Ca^{2+} (53), whereas the Ca^{2+} concentration within endosomes is lower by 100- to 1000-fold (54). This loss of the stabilizing calcium ion in endosomes could reduce the affinity of the internalized CD23 for IgE, causing the release of the bound IgE-allergen complex “cargo.”

derCD23 BINDS ASYMMETRICALLY TO IgE-Fc

Studies on the binding of CD23 to the complete Fc region of IgE show a more complex picture than for CD23 bound to the Fc ϵ 3–4 fragment (described above). The binding of derCD23 to IgE-Fc confirmed the 2:1 stoichiometry previously established for binding to the Fc ϵ 3–4 fragment (47, 48), and the affinities of the two binding sites differed by an order of magnitude ($K_{D1} = 1.2 \times 10^{-6}$ M and $K_{D2} = 1.4 \times 10^{-5}$ M). The crystal structure of IgE-Fc in complex with derCD23 (48) showed only one derCD23 molecule bound, to the heavy chain onto which the C ϵ 2 domains are folded back (**Figure 2d**). In order to accommodate the binding of the derCD23 molecule the C ϵ 2 domain pair swing out by 16° , resulting in a slight “unbending” of IgE-Fc.

As a result of the 1:1 stoichiometry observed in the IgE-Fc/derCD23 crystal structure, the C ϵ 3–C ϵ 4 chain to which derCD23 is bound adopts an extremely closed conformation as expected, whereas the free chain has a very open conformation which is incompatible with binding of derCD23. It is clear that a conformational change in the free heavy chain of IgE-Fc is required to allow it to bind the second derCD23 molecule, consistent with the observation of substantially weaker affinity for the second CD23 binding event (47, 48).

It seems most likely that the derCD23 molecule seen in this structure binds at the higher-affinity site indicated by the ITC determination. If so, this could involve the concomitant slight unbending of IgE-Fc and modulation of the affinity of the second binding site. Thus, the IgE-Fc structural and biophysical data may suggest a mechanism of “cross talk” between the two CD23-binding sites of IgE. This is intriguing, as a reciprocal allosteric inhibition in which conformational changes in IgE upon CD23 binding inhibit the binding of Fc ϵ RI and *vice versa*, and local conformational changes as a result of Ca^{2+} binding to CD23 enhance IgE binding have been established.

Together, these mechanisms suggest an intricate level of control of the interactions of the IgE network that could regulate diverse aspects of the allergic response.

CD21 BINDS TO THE LECTIN HEAD DOMAIN OF CD23

CD21 (also known as CR2) is a glycoprotein, comprising 15 or 16 short consensus repeat (SCR) domains, a common motif in a variety of different proteins. Small angle X-ray and neutron solution scattering, along with ultracentrifugation modelling (55), show that the 15 SCR domains are flexible and partially folded back. The crystal structure of CD21 D1-D2 has been determined (56) (**Figure 1i**). Despite extensive contacts at the interface between the two domains, there is substantial interdomain flexibility, with D1 forming an open V-shaped structure with D2 (55).

The CD21 D1-D2 binding site on derCD23 has been mapped by NMR chemical shift perturbation studies to residues of the C-terminal “tail,” with SPR studies demonstrating an affinity of $K_D = 8.7 \times 10^{-7}$ M (31).

It is clear that the human CD23 CTLD cannot act as a traditional CRD, and so some aspects of the CD23/CD21 interaction mechanism remain unelucidated. Indeed, there is a paucity of structural and biophysical information on CD21 and its interactions with CD23 and the greater IgE network, which are critical in the regulation of IgE synthesis.

A MODEL FOR IgE HOMEOSTASIS

The complex interactions between members of the IgE network have led to a model for IgE homeostasis, involving a two-way switch, for the upregulation and downregulation of IgE. At low IgE concentrations, membrane-bound CD23, unprotected from cleavage by an endogenous protease, ADAM 10, is cleaved from the B cell surface and the resulting soluble CD23 trimers co-ligate membrane IgE and CD21 to stimulate IgE synthesis in IgE-expressing (switched) B cells. This mechanism is similar to the process of upregulation of B cell antibody synthesis by the co-ligation of CD21 with the B cell receptor (BCR) by antigen covalently linked to the complement fragment C3d (57, 58). At high concentrations of IgE, however, excess IgE or antigen-IgE complexes bind to membrane CD23 on B cells, protecting it from proteolytic cleavage, terminating further IgE production (**Supplemental Figure 1**). The binding sites for IgE and CD21 on CD23, mapped by chemical perturbation, are distant from each other (31); IgE binds at a site proximal to loops 1 and 4 of the CD23 lectin head domain and CD21 in the C-terminal tail region. Furthermore, it has been shown that a heterotrimeric complex of IgE-Fc, derD23, and CD21 (D1-D2) can form in vitro (31). Compelling functional evidence for the IgE homeostasis hypothesis has been reported from in vitro experiments by adding monomeric and trimeric recombinant fragments of CD23 to IL-4-stimulated human PBMC in vitro (29, 30). Mouse CD23 lacks the C-terminal tail found in human CD23 and is therefore able to manifest only the downregulation of IgE production by trimeric CD23 via membrane IgE. The repurposing of CD21 for IgE up-regulation likely involved a new branch of CD23 evolution in humans. This mechanism is reviewed in greater detail in the context of structure-function relationships of human CD21 (59).

Supplemental Material >

ANTI-IgE BIOLOGICS

There was no consensus that IgE is central to asthma until the effectiveness of anti-IgE therapies in the treatment of asthma provided “proof of principle” (60). A new recognition that asthma is a heterogeneous disease has focused recent research on improving the efficacy and inhibitory properties of anti-IgE antibodies along with other approaches for personalized medicine. In turn, this has illuminated various mechanisms by which the functions of IgE can be modulated.

Structural determination of IgE-Fc (or Fcε3-4) complexed to anti-IgEs has often been a starting point for the elucidation of mechanism of action.

Omalizumab

The value of targeting IgE for therapy is demonstrated by the success of the humanized IgG1κ anti-IgE monoclonal antibody (mAb) omalizumab (Xolair; Novartis Pharmaceuticals Ltd), in the treatment of asthma and other allergic diseases (61). Moreover, anti-IgE-mediated reduction in free IgE levels results in the reduction in the number of FcεRI receptors on mast cells, basophils, and antigen-presenting cells (62).

It had been widely reported (*i*) that omalizumab and FcεRI bind the same site on IgE and (*ii*) that omalizumab cannot bind to IgE when it is bound to FcεRI (63, 64). As described below,

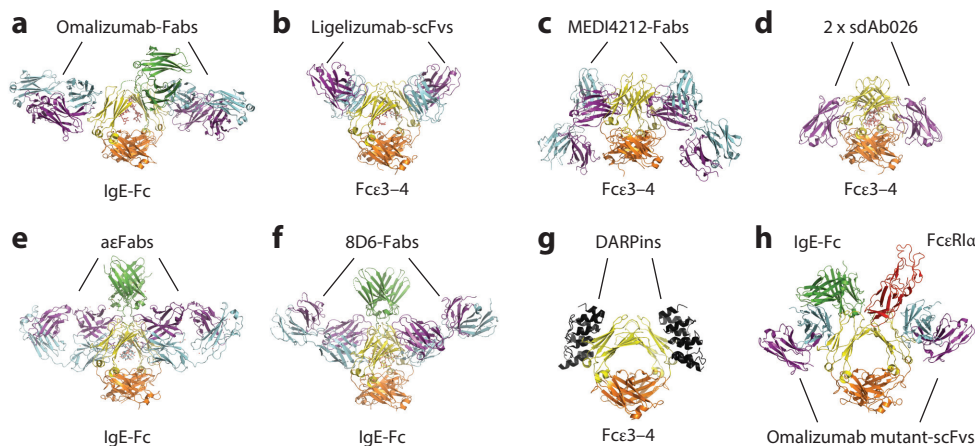


Figure 4

IgE/anti-IgE biologic complexes. (a) Crystal structure of IgE-Fc in complex with omalizumab-Fab (PDB ID 5G64). (b) Fcε3-4/ligelizumab-scFv (PDB ID 6UQR). (c) Fcε3-4/MEDI4212-Fab (PDB ID 5ANM). (d) Fcε3-4/sdAb026 (PDB ID 5NQW). (e) IgE-Fc/αεFab (PDB ID 4J4P). (f) IgE-Fc/8D6-Fab (PDB ID 6EYO). (g) IgE-Fc/DARPin (PDB ID 4GRG). (h) Cryo-EM structure of the IgE-Fc/sFcεRIα/omalizumab mutant-scFv complex (PDB ID 7SHT). The Fab fragment heavy and light chains are colored purple and cyan, respectively. Abbreviations: cryo-EM, cryo-electron microscopy; DARPin, designed ankyrin repeat protein; PDB ID, Protein Data Bank identifier.

both of these claims have proven to be incorrect. The mechanism by which omalizumab inhibits the binding of IgE to both FcεRI and CD23 is illuminated by the crystal structures of omalizumab Fabs bound to either a constrained Fcε3-4 molecule with the mutation G335C, which locks the Cε3 domains in a closed conformation (65), or intact IgE-Fc, including the Cε2 domains (66). The latter complex is shown in **Figure 4a**. Both structures show two omalizumab Fabs bound, one to each Cε3 domain, but in contrast to the complex with Fcε3-4(G335C) and the disulfide-tethered Cε3 domains, in the complex with IgE-Fc, the Cε3 domains undergo a substantial “opening up” (66). In fact, the Cε3 domains in the omalizumab-Fab/IgE-Fc complex are so far apart that FcεRI cannot engage both subsites simultaneously, and thus high-affinity binding is lost. The binding of the two Fabs to IgE-Fc also involves an “unbending” of the Fc, with the (Cε2)₂ domain pair rotating away from the Fcε3-4 region and the acutely bent conformation observed in both free IgE-Fc (4) and FcεRI-bound IgE-Fc (14). One of the two omalizumab Fabs makes a few contacts with one of the Cε2 domains (**Figure 4a**). Thus omalizumab binding causes substantial conformational changes in IgE-Fc, in particular the disruption of the high-affinity FcεRI binding site through increased separation of the two Cε3 domains: inhibition of FcεRI binding by omalizumab is thus allosteric. In contrast, both complex structures agree that inhibition of CD23 binding is orthosteric, since there is considerable overlap between the space occupied by the bound omalizumab Fabs and that occupied by CD23 (45), as well as overlap between Cε3 domain residues involved in binding both ligands.

FRET experiments have shown that in solution, omalizumab Fab binding partially “unbends” the IgE-Fc (66), and solution studies also confirm the allosteric nature of the inhibition of IgE-Fc binding to FcεRI binding by omalizumab (66). The ability of omalizumab to not only inhibit FcεRI binding, but also induce dissociation of FcεRI-bound IgE, will be discussed below.

Ligelizumab

Ligelizumab (Novartis Pharmaceuticals Ltd.) is another humanized IgG1κ anti-IgE mAb currently in development with aspirations for therapeutic use (61). Similar to omalizumab, the

ligelizumab single-chain variable fragments (scFv) contact symmetrical binding sites on the two Cε3 domains, with the Fcε3–4 adopting an open conformation (67) (**Figure 4b**). Strikingly, however, the IgE epitopes for ligelizumab and omalizumab only partially overlap: the ligelizumab epitope overlaps more completely with the FcεRI binding-site on IgE than does omalizumab. But, as ligelizumab shares *no* overlap with the CD23 binding-site on IgE, ligelizumab is far less efficient than omalizumab at inhibiting the CD23/IgE interaction. Moreover, the affinity of the ligelizumab-Fab/full-length IgE interaction ($K_D = 3.5 \times 10^{-11}$ M) is approximately 100-fold greater than that of the interaction of omalizumab-Fab with full-length IgE (67).

MEDI4212

The antibody MEDI4212 exhibits higher affinity for IgE than omalizumab ($K_D = 1.95 \times 10^{-12}$ M) (68), the advantages of which promised to allow the treatment of patients with either IgE concentrations in blood or body mass over the limit and other subgroups of patients of significant proportions found to be nonresponders to omalizumab. The MEDI4212-Fab/Fcε3–4 complex crystal structure revealed a 2:1 stoichiometry, with the MEDI4212 epitope partially overlapping the FcεRI binding site (**Figure 4c**). In addition, MEDI4212 binds to and stabilizes the open conformation of Fcε3–4. This anti-IgE biologic displayed significantly more efficient inhibition of FcεRI binding to IgE and mast cell degranulation by allergens than omalizumab *in vitro*.

Immunoglobulin Single-Variable Domain 026

An immunoglobulin single variable domain (sdab), called “sdab026,” has been designed to target IgE. It binds with a dissociation constant $K_D = 1.4 \times 10^{-9}$ M. The 2:1 stoichiometric sdab026/IgE-Fc complex crystal structure reveals that the sdab026 epitope significantly overlaps the CD23-binding site on IgE, but only slightly overlaps with the FcεRI-binding site (69) (**Figure 4d**). However, the Fcε3–4 moiety is observed to adopt an extremely closed conformation upon sdab026 binding; hence, the sdab is able to inhibit both FcεRI (allosterically) and CD23 (orthosterically) binding to IgE, and abrogate both basophil activation and facilitated antigen binding *in vitro*. Small angle X-ray scattering studies indicate that sdab026 binding induces a slight unbending of IgE-Fc (69).

aeFab and Complete IgE-Fc Unbending

An anti-IgE IgG-Fab was generated, called aeFab. Upon elucidation of the IgE-Fc/aeFab complex crystal structure a 2:1 stoichiometry of aeFab binding to IgE was determined (70). It was a complete surprise when the Cε2 domain pair were observed to adopt a fully extended conformation relative to the Fcε3–4 region, i.e., a *complete* “unbending” of the IgE-Fc (**Figure 4e**).

The aeFab light and heavy chains bind to diametrically opposed, structurally equivalent, sites on each heavy chain of IgE-Fc. The interaction mainly involves Cε3, with a small contribution from Cε2. The conformational change in the molecule is driven by large movements of the Cε2–Cε3 linker, resulting in a complete unbending of IgE-Fc by 120° compared to unliganded IgE-Fc.

As mentioned previously, the data for earlier FRET studies (23), small angle X-ray scattering measurements (22) and X-ray crystallography structures (4, 14) were consistent with a bent IgE-Fc conformation. So, the question remained whether this extended IgE-Fc structure could be adopted in the absence of aeFab. Stopped-flow kinetic and ITC studies are consistent with a “conformational selection” model, with extended structures selected from a dynamic population of IgE-Fc conformations. Moreover, molecular dynamic simulations and free energy calculations predict that the Cε2 domain pair can “flip” from one side of the Fcε3–4 moiety to the other, via a transient fully extended (Cε2)₂ conformational intermediate (70).

A low resolution cryo-EM structure of intact IgE bound to ligelizumab-Fab has recently been elucidated (24). It reveals that ligelizumab binding can also result in an extended conformation of IgE.

8D6

The antihuman IgE antibody 8D6 is a remarkable anti-IgE in several respects. The crystal structure of the 8D6-Fab/IgE-Fc complex reveals an almost fully extended IgE-Fc with rotational symmetry and 2:1 stoichiometry (71) (**Figure 4f**). However, the conformation of IgE-Fc in the complex is more compact than that observed in the IgE-Fc/ α Fab complex. The C ϵ 2 of chain B separates from C ϵ 3 and C ϵ 4 of chain A, twisting around C ϵ 2-C ϵ 3 linkers as a unit to bind to the two individual C ϵ 3 domains in a novel way.

An important functional difference from the other anti-IgEs discussed above is that 8D6 inhibits only Fc ϵ RI binding to IgE, but allows CD23 to bind with the same affinity, whether to free IgE or 8D6/IgE complex (71).

Anti-IgE DARPin

Beyond the immunoglobulin fold as a therapeutic modality, alternative anti-IgE molecular scaffolds have been generated. For example, designed ankyrin repeat proteins (DARPin) are derived from the ankyrin repeat motif present in numerous naturally occurring proteins. Ankyrin repeat-containing proteins are involved in a wide variety of biological activities (72).

An IgE-directed DARPin engineered inhibitor, E2_79, has been shown to inhibit the IgE/Fc ϵ RI interaction (73). Using crystallographic, mutational, fluorescence, SPR and quantitative kinetic modelling techniques, a nonclassical inhibitory mechanism was proposed.

The E2_79/ Fc ϵ 3-4 complex crystal structure reveals that E2_79 binds with a 2:1 stoichiometry to the closed form of Fc ϵ 3-4, binding to each C ϵ 3 domain (**Figure 4g**). Superposition of the complex with the asymmetric IgE-Fc/sFc ϵ RI α crystal structure demonstrated that one of the bound E2_79 molecules is remote from either Subsite 1 or 2 (see the section titled Structures of sFc ϵ RI α and Its Complex with sFc ϵ RI α and Fc ϵ 3-4 Enabled a Preliminary Understanding of the IgE-Fc ϵ RI Interaction), while the other E2_79 engages Fc ϵ 3-4 near Subsite 2, resulting in partial steric overlap of the DARPin and sFc ϵ RI α .

INDUCED DISSOCIATION OF IgE

Here, we use the term “induced dissociation” generically, without prejudice regarding the mechanism employed, to describe the phenomenon of one ligand increasing the dissociation rate of a preformed receptor/ligand complex, compared to the dissociation rate of the bimolecular receptor/ligand complex in the absence of the competing ligand. In the literature describing related phenomena, the term “induced” might be replaced with “accelerated”, “assisted”, “facilitated” or “ligand-mediated”, and “dissociation” might instead be described as “unbinding”, “displacement”, “disruption”, “disassembly” or “release.” None of these different terms are used consistently across the literature to differentiate distinct mechanisms.

IgE's capacity for induced dissociation has been fully appreciated only recently. In 2002, Miyajima et al. (74) described a rat monoclonal anti-IgE antibody that removed IgE bound to Fc ϵ RI on mast cells, resulting in inhibition of anaphylaxis in animal models. Around 2010, the Belgian antibody company Ablynx was pursuing an anti-IgE program (75), and identified a single domain anti-IgE antibody, ALX-0962, that could displace IgE bound to Fc ϵ RI, as demonstrated by surface plasmon resonance experiments and functionally in RBL degranulation assays (76). In 2012, researchers from Stanford University and the University of Bern first described E2_79, a DARPin-based anti-IgE that could disrupt preformed IgE-Fc ϵ RI complexes (73).

It was later noted that the therapeutic anti-IgE omalizumab (Xolair) could also induce dissociation of IgE from FcεRI (77), although inefficiently and only at concentrations much higher than those used therapeutically (78). However, modified versions of the omalizumab Fab, optimized for these disruptive properties, were shown to rapidly and efficiently dissociate IgE from FcεRI (66, 79). Anti-IgE DARPins have also been engineered for marked improvements in their ability to induce dissociation of IgE/FcεRI complexes (80).

The mechanisms of induced dissociation in IgE have not been fully elucidated, partly because the mechanisms used by different molecules are likely to be somewhat different. Different mechanisms have been proposed (66, 73). In the broadest sense, one could imagine a number of distinct mechanisms by which the process of induced dissociation could occur. For example, molecular competition events that occur through proximal competing ligands, i.e., proximity-induced dissociation processes, or competition occurring via distal molecules, i.e., allosterically-induced dissociation mechanisms. Observations of ligand-dependent acceleration of dissociation rates have also been attributed to prevention of rapid ligand rebinding events (81). Combinations of these different mechanisms could contribute to induced dissociation processes in some systems. The use of antibodies or antibody fragments to characterize proximity-induced dissociation was recently described by Abdiche et al. (82). In this study, data are presented for a number of antibody/antigen systems, consistent with the hypothesis that antibodies with closely adjacent or minimally overlapping epitopes, can mediate induced dissociation. The mechanism requires the formation of a transient trimolecular complex of two proximal ligands (for example, see **Figure 4b** and Reference 79), which allows the incoming second ligand to perturb the first. In contrast, too much overlap of antibody epitopes prevents this phenomenon because the trimolecular complex cannot form; this situation is instead traditional orthosteric competition between two ligands.

Ligand-induced dissociation is known to occur in a wide variety of important biological processes. To cite just a few examples, these processes have been shown to occur in ribosomal elongation and release factors (83, 84), in the insulin/IGF-1 interaction (85), for inhibitors of TNF-α (86), and in a large number of DNA/transcription factor complexes (87). While some of these systems have been well characterized kinetically, detailed structural and mechanistic understanding is often lacking.

Within the IgE system, attention has understandably focused on disruption of IgE/FcεRI complexes, but induced dissociation seems to be a general feature of the IgE molecule. This is at least partly because IgE's intrinsic conformational dynamics and capacity for allosteric modulation makes the molecule very well suited to ligand competition processes. In addition to FcεRI, other IgE ligands have been shown to be susceptible to induced dissociation. For example, some but not all of the anti-IgE antibodies described above show these effects. Because IgE-Fc is a homodimer, most anti-IgE antibodies bind with a 2:1 (anti-IgE Ab:IgE) stoichiometry. Like the binding of derCD23 to IgE (45), anti-IgE antibodies usually bind asymmetrically, with one binding site showing higher affinity than the second. To cite two examples, the binding of the second αFab molecule destabilizes the αFab/IgE-Fc complex (70), and the second omalizumab Fab induces the dissociation of IgE-Fc from a 1:1 omalizumab Fab/IgE-Fc complex (66). Because the binding sites for these Fab pairs are far apart from each other on the IgE dimer, induced dissociation of these ligands can only occur through an allosteric mechanism. For a number of the characterized IgE/FcεRI disruptors, the mechanisms are less clear. Kim et al. (73) describe the mechanism of the DARPin E2_79 as facilitated dissociation (as defined by Reference 88) and suggest that classic allosteric mechanisms cannot fully explain the observed dissociative effects; this model has some of the general characteristics of proximity-induced dissociation, and X-ray crystal structures are consistent with the potential for proximity-mediated effects. Modified versions of omalizumab

Fabs clearly show mixed inhibitory mechanisms, with clear evidence of allosteric mechanisms but with one of the binding sites sufficiently close to the FcεRI binding site that proximity effects are possible (66).

IgE has undoubtedly undergone evolutionary selection for these properties. As noted above, the mutually inhibitory allosteric competition between FcεRI and CD23 is essential for preventing spontaneous anaphylaxis (46). We speculate that there are other advantages to IgE's capacity for induced dissociation in the natural function of IgE, as this allows opportunities for greater dynamic control of the activities of IgE and IgE/ligand complexes.

There are also clearly opportunities to exploit these properties for therapeutic effect. For example, the therapeutic anti-IgE omalizumab has been an effective treatment for asthma patients (60), but it is very slow to show clinical effect (89). Under the concentrations used in the clinic, omalizumab does not dissociate IgE from FcεRI on mast cells, but it does bind to any unbound IgE and blocks these IgE molecules from binding to effector cells. Still, there remains a substantial population of IgE bound to mast cells, which can mediate allergic reactions. It takes weeks for this IgE to dissociate naturally, and this undoubtedly contributes to omalizumab's slow therapeutic effect. An anti-IgE that efficiently induced dissociation of IgE from FcεRI might reduce the time required for therapeutic benefit from weeks to hours.

THE GENERAL CHARACTERISTICS OF IgE ALLOSTERY

IgE-Fc has a number of structural and physical properties that underpin its allosteric capacity. Structural studies of IgE-Fc and IgE-Fc/ligand complexes have shown that IgE-Fc can exist in multiple conformations (4, 13, 68–70), indeed that it is capable of undergoing strikingly large-scale structural changes (70, 71). Free energy calculations predict that the energy differences between these different conformations can be large, but perhaps not too large, and the energy barriers separating these states are not insurmountable (70). Effective allosteric systems rely on these Goldilocks-like characteristics for the energy differences between conformers and the energy barriers between them. This allows ligands to either induce or select conformations, to overcome modest energy barriers, and to alter conformational energies that change the equilibria of favored structural states.

IgE-Fc's allosteric capacity is further potentiated by its homodimeric structure. For IgE ligands with the potential to bind with 2:1 stoichiometry to the homodimer, the initial molecule that binds will almost always introduce structural asymmetry to the dimer. For a molecule with IgE's structural plasticity, this may manifest itself as conformational changes, either large-scale changes through long-range rigid body motions from the reorientation of domains, or more local structural changes of the atypically dynamic Cε3 domains or interdomain linker regions, or more subtle alterations in structural dynamics, which can then affect other ligand binding events through entropy changes (90).

IgE's structural heterogeneity is not limited only to unbound forms of IgE. Several research groups have experimentally observed heterogeneity in how IgE binds FcεRI (91–94). That is, IgE does not bind to FcεRI with a single affinity; different binding populations are observed. So IgE's structural heterogeneity translates into functional heterogeneity. There are different ways that IgE can bind to FcεRI, some with the very slow off-rates we associate with interaction of the “high-affinity IgE receptor”, but within this ensemble of possible bound conformations some are intrinsically less stable. Allosterically-induced dissociation in IgE most likely works by favoring the more weakly binding conformations, leading to markedly destabilized complexes. Even proximity-induced dissociation is likely to be more efficient in a structurally dynamic complex, as it is difficult to imagine this process occurring in a completely structurally rigid system.

SUMMARY AND OUTLOOK

Once a consensus was reached that IgE is fundamental to asthma (60), urticaria (95), and allergic disorders more generally, it was obvious that intervention in the expression or action of IgE would be a direct way to alleviate the development and course of these diseases.

We have reviewed the structures and mechanisms of action of a network of proteins involved in the pathogenesis of allergy and urticaria, including IgE and its cell receptors, FcεRI, CD23, and coreceptor CD21. IgE has a unique conformational plasticity, which enables it to bind either to FcεRI to exert its effector functions or to CD23 to control IgE production and homeostasis. These interactions are mutually antagonistic because the receptors bind to different IgE conformations, in which the Cε3 domains, to which they bind, have either an “open” (FcεRI) or “closed” (CD23) conformation. The intricate mechanisms underlying these conformational transitions have been elucidated by structural, biophysical and thermodynamic studies, discussed in this review. They show the importance of Cε2, which is unique to IgE among the switched isotypes, and its role in the degree of unbending induced by receptor or anti-IgE antibodies binding to Cε3.

The long-range effects of antigen binding to IgE and antigen cross-linking of the IgE-receptor complexes on effector cells or membrane IgE on B cells are complex and still not fully understood, inviting future research. Further study is also required to understand the structure of various multiprotein complexes, and to establish in vivo the contribution of CD21 studied to date only in vitro, and pursue further the proposed mechanism of IgE homeostasis.

Elucidation of aspects of IgE structure, dynamics, allostery, ligand interactions and induced dissociation has given insights into novel mechanisms of IgE regulation. Knowledge of the mechanisms described here have identified new targets for intervention, for example allowing discovery of agents that trap IgE in inactive conformations, or promote IgE dissociation from its receptors. However, our understanding of IgE in all its complexity is far from complete.

DISCLOSURE STATEMENT

J.M.M. and B.J.S. are inventors on a patent for anti-IgE antibodies (US11214627B2). H.J.G. is an inventor on a patent for IgE antibodies against cancer targets (WO2021214329).

ACKNOWLEDGMENTS

The authors acknowledge support by Asthma UK (AUK-09/029), the Biotechnology and Biological Sciences Research Council UK (BB/P000436, BB/K501815), the Medical Research Council UK (G0501494, G1100090), and the Wellcome Trust (076343).

LITERATURE CITED

1. Gould HJ, Sutton BJ. 2008. IgE in allergy and asthma today. *Nat. Rev. Immunol.* 8(3):205–17
2. Sutton BJ, Davies AM. 2015. Structure and dynamics of IgE-receptor interactions: FcεRI and CD23/FcεRII. *Immunol. Rev.* 268(1):222–35
3. Sutton BJ, Davies AM, Bax HJ, Karagiannis SN. 2019. IgE antibodies: from structure to function and clinical translation. *Antibodies* 8(1):E19
4. Wan T, Beavil RL, Fabiane SM, Beavil AJ, Sohi MK, et al. 2002. The crystal structure of IgE Fc reveals an asymmetrically bent conformation. *Nat. Immunol.* 3(7):681–86
5. Price NE, Price NC, Kelly SM, McDonnell JM. 2005. The key role of protein flexibility in modulating IgE interactions. *J. Biol. Chem.* 280(3):2324–30
6. Harwood NE, McDonnell JM. 2007. The intrinsic flexibility of IgE and its role in binding FcεRI. *Biomed. Pharmacother.* 61(1):61–67
7. Kinet JP. 1999. The high-affinity IgE receptor (FcεRI): from physiology to pathology. *Annu. Rev. Immunol.* 17:931–72

8. Garman SC, Kinet JP, Jardetzky TS. 1998. Crystal structure of the human high-affinity IgE receptor. *Cell* 95(7):951–61
9. Mallamaci MA, Chizzonite R, Griffin M, Nettleton M, Hakimi J, et al. 1993. Identification of sites on the human Fc epsilon RI alpha subunit which are involved in binding human and rat IgE. *J. Biol. Chem.* 268(29):22076–83
10. Keown MB, Ghirlando R, Mackay GA, Sutton BJ, Gould HJ. 1997. Basis of the 1:1 stoichiometry of the high affinity receptor Fc epsilon RI-IgE complex. *Eur. Biophys. J.* 25(5–6):471–76
11. Garman SC, Wurzburg BA, Tarchevskaya SS, Kinet JP, Jardetzky TS. 2000. Structure of the Fc fragment of human IgE bound to its high-affinity receptor FcεRIα. *Nature* 406(6793):259–66
12. Radaev S, Sun P. 2002. Recognition of immunoglobulins by Fcγ receptors. *Mol. Immunol.* 38(14):1073–83
13. Wurzburg BA, Jardetzky TS. 2009. Conformational flexibility in immunoglobulin E-Fc₃₋₄ revealed in multiple crystal forms. *J. Mol. Biol.* 393(1):176–90
14. Holdom MD, Davies AM, Nettleship JE, Bagby SC, Dhaliwal B, et al. 2011. Conformational changes in IgE contribute to its uniquely slow dissociation rate from receptor FcεRI. *Nat. Struct. Mol. Biol.* 18(5):571–76
15. Wurzburg BA, Garman SC, Jardetzky TS. 2000. Structure of the human IgE-Fc Cε3–Cε4 reveals conformational flexibility in the antibody effector domains. *Immunity* 13(3):375–85
16. Doré KA, Davies AM, Drinkwater N, Beavil AJ, McDonnell JM, Sutton BJ. 2017. Thermal sensitivity and flexibility of the Cε3 domains in immunoglobulin E. *Biochim. Biophys. Acta Proteins Proteom.* 1865(11 Part A):1336–47
17. Borthakur S, Andrejeva G, McDonnell JM. 2011. Basis of the intrinsic flexibility of the Cε3 domain of IgE. *Biochemistry* 50(21):4608–14
18. Feige MJ, Walter S, Buchner J. 2004. Folding mechanism of the C_H2 antibody domain. *J. Mol. Biol.* 344(1):107–18
19. Fink AL. 2005. Natively unfolded proteins. *Curr. Opin. Struct. Biol.* 15(1):35–41
20. Harwood NE, Price NC, McDonnell JM. 2006. Catalytic folding of the Cε3 domain by its high affinity receptor. *FEBS Lett.* 580(8):2129–34
21. McDonnell JM, Calvert R, Beavil RL, Beavil AJ, Henry AJ, et al. 2001. The structure of the IgE Cε2 domain and its role in stabilizing the complex with its high-affinity receptor FcεRIα. *Nat. Struct. Biol.* 8(5):437–41
22. Beavil AJ, Young RJ, Sutton BJ, Perkins SJ. 1995. Bent domain structure of recombinant human IgE-Fc in solution by X-ray and neutron scattering in conjunction with an automated curve fitting procedure. *Biochemistry* 34(44):14449–61
23. Zheng Y, Shopes B, Holowka D, Baird B. 1992. Dynamic conformations compared for IgE and IgG1 in solution and bound to receptors. *Biochemistry* 31(33):7446–56
24. Jensen RK, Jabs F, Mische M, Mølgaard B, Pützner W, et al. 2020. Structure of intact IgE and the mechanism of ligelizumab revealed by electron microscopy. *Allergy* 75(8):1956–65
25. Zelensky AN, Gready JE. 2005. The C-type lectin-like domain superfamily. *FEBS J.* 272(24):6179–217
26. Kijimoto-Ochiai S. 2002. CD23 (the low-affinity IgE receptor) as a C-type lectin: a multidomain and multifunctional molecule. *Cell Mol. Life Sci.* 59(4):648–64
27. Natarajan K, Dimasi N, Wang J, Mariuzza RA, Margulies DH. 2002. Structure and function of natural killer cell receptors: multiple molecular solutions to self, nonself discrimination. *Annu. Rev. Immunol.* 20:853–85
28. Beavil AJ, Edmeades RL, Gould HJ, Sutton BJ. 1992. Alpha-helical coiled-coil stalks in the low-affinity receptor for IgE (Fc epsilon RII/CD23) and related C-type lectins. *PNAS* 89(2):753–57
29. Cooper AM, Hobson PS, Jutton MR, Kao MW, Drung B, et al. 2012. Soluble CD23 controls IgE synthesis and homeostasis in human B cells. *J. Immunol.* 188(7):3199–207
30. McCloskey N, Hunt J, Beavil RL, Jutton MR, Grundy GJ, et al. 2007. Soluble CD23 monomers inhibit and oligomers stimulate IGE synthesis in human B cells. *J. Biol. Chem.* 282(33):24083–91
31. Hibbert RG, Teriete P, Grundy GJ, Beavil RL, Reljic R, et al. 2005. The structure of human CD23 and its interactions with IgE and CD21. *J. Exp. Med.* 202(6):751–60
32. Weskamp G, Ford JW, Sturgill J, Martin S, Docherty AJP, et al. 2006. ADAM10 is a principal “shedase” of the low-affinity immunoglobulin E receptor CD23. *Nat. Immunol.* 7(12):1293–98

33. Weis WI, Taylor ME, Drickamer K. 1998. The C-type lectin superfamily in the immune system. *Immunol. Rev.* 163:19–34
34. Marcelletti JF, Richards ML, Katz DH. 1989. The emerging importance of IgE and Fc receptors for IgE (Fc epsilon R) in the regulation of B cell activity. *Contrib. Microbiol. Immunol.* 11:188–205
35. Vercelli D, Helm B, Marsh P, Padlan E, Geha RS, Gould H. 1989. The B-cell binding site on human immunoglobulin E. *Nature* 338(6217):649–51
36. Aubry JP, Pochon S, Graber P, Jansen KU, Bonnefoy JY. 1992. CD21 is a ligand for CD23 and regulates IgE production. *Nature* 358(6386):505–7
37. Szakonyi G, Guthridge JM, Li D, Young K, Holers VM, Chen XS. 2001. Structure of complement receptor 2 in complex with its C3d ligand. *Science* 292(5522):1725–28
38. Nemerow GR, Houghten RA, Moore MD, Cooper NR. 1989. Identification of an epitope in the major envelope protein of Epstein-Barr virus that mediates viral binding to the B lymphocyte EBV receptor (CR2). *Cell* 56(3):369–77
39. Delcayre AX, Salas F, Mathur S, Kovats K, Lotz M, Lernhardt W. 1991. Epstein Barr virus/complement C3d receptor is an interferon alpha receptor. *EMBO J.* 10(4):919–26
40. Wurzburg BA, Tarchevskaya SS, Jardetzky TS. 2006. Structural changes in the lectin domain of CD23, the low-affinity IgE receptor, upon calcium binding. *Structure* 14(6):1049–58
41. Bettler B, Texido G, Raggini S, Rüegg D, Hofstetter H. 1992. Immunoglobulin E-binding site in Fc epsilon receptor (Fc epsilon RII/CD23) identified by homolog-scanning mutagenesis. *J. Biol. Chem.* 267(1):185–91
42. Hoppe HJ, Reid KB. 1994. Trimeric C-type lectin domains in host defence. *Structure* 2(12):1129–33
43. Yuan D, Keeble AH, Hibbert RG, Fabiane S, Gould HJ, et al. 2013. Ca²⁺-dependent structural changes in the B-cell receptor CD23 increase its affinity for human immunoglobulin E. *J. Biol. Chem.* 288(30):21667–77
44. Dhaliwal B, Pang MOY, Yuan D, Yahya N, Fabiane SM, et al. 2013. Conformational plasticity at the IgE-binding site of the B-cell receptor CD23. *Mol. Immunol.* 56(4):693–97
45. Dhaliwal B, Yuan D, Pang MOY, Henry AJ, Cain K, et al. 2012. Crystal structure of IgE bound to its B-cell receptor CD23 reveals a mechanism of reciprocal allosteric inhibition with high affinity receptor FcεRI. *PNAS* 109(31):12686–91
46. Borthakur S, Hibbert RG, Pang MOY, Yahya N, Bax HJ, et al. 2012. Mapping of the CD23 binding site on immunoglobulin E (IgE) and allosteric control of the IgE-FcεRI interaction. *J. Biol. Chem.* 287(37):31457–61
47. Shi J, Ghirlando R, Bevil RL, Bevil AJ, Keown MB, et al. 1997. Interaction of the low-affinity receptor CD23/FcεRII lectin domain with the Fcε3–4 fragment of human immunoglobulin E. *Biochemistry* 36(8):2112–22
48. Dhaliwal B, Pang MOY, Keeble AH, James LK, Gould HJ, et al. 2017. IgE binds asymmetrically to its B cell receptor CD23. *Sci. Rep.* 7:45533
49. Kelly AE, Chen BH, Woodward EC, Conrad DH. 1998. Production of a chimeric form of CD23 that is oligomeric and blocks IgE binding to the FcεRI. *J. Immunol.* 161(12):6696–704
50. Jégouzo SAF, Feinberg H, Morrison AG, Holder A, May A, et al. 2019. CD23 is a glycan-binding receptor in some mammalian species. *J. Biol. Chem.* 294(41):14845–59
51. Ilkow VF, Davies AM, Dhaliwal B, Bevil AJ, Sutton BJ, McDonnell JM. 2021. Reviving lost binding sites: exploring calcium-binding site transitions between human and murine CD23. *FEBS Open Biol.* 11(7):1827–40
52. Karagiannis SN, Warrack JK, Jennings KH, Murdock PR, Christie G, et al. 2001. Endocytosis and recycling of the complex between CD23 and HLA-DR in human B cells. *Immunology* 103(3):319–31
53. Ohman S, Larsson L. 1978. Higher values for ionized calcium with a new type of electrode for Orion SS-20. *Clin. Chem.* 24(11):2070–71
54. Andersen CBF, Moestrup SK. 2014. How calcium makes endocytic receptors attractive. *Trends Biochem. Sci.* 39(2):82–90
55. Gilbert HE, Asokan R, Holers VM, Perkins SJ. 2006. The 15 SCR flexible extracellular domains of human complement receptor type 2 can mediate multiple ligand and antigen interactions. *J. Mol. Biol.* 362(5):1132–47

56. Prota AE, Sage DR, Stehle T, Fingerroth JD. 2002. The crystal structure of human CD21: implications for Epstein-Barr virus and C3d binding. *PNAS* 99(16):10641–46
57. Carter RH, Fearon DT. 1992. CD19: lowering the threshold for antigen receptor stimulation of B lymphocytes. *Science* 256(5053):105–7
58. Dempsey PW, Fearon DT. 1996. Complement: instructing the acquired immune system through the CD21/CD19 complex. *Res. Immunol.* 147(2):71–75; discussion 119–120
59. Hannan JP. 2016. The structure-function relationships of complement receptor type 2 (CR2; CD21). *Curr. Protein Pept. Sci.* 17(5):463–87
60. Babu KS, Holgate ST. 2002. The role of anti-IgE therapies in the treatment of asthma. *Hosp. Med.* 63(8):483–86
61. Guntern P, Eggel A. 2020. Past, present, and future of anti-IgE biologics. *Allergy* 75(10):2491–502
62. Beck LA, Marcotte GV, MacGlashan D, Togias A, Saini S. 2004. Omalizumab-induced reductions in mast cell FcεRI expression and function. *J. Allergy Clin. Immunol.* 114(3):527–30
63. Vichyanond P. 2011. Omalizumab in allergic diseases, a recent review. *Asian Pac. J. Allergy Immunol.* 29(3):209–19
64. Schulman ES. 2001. Development of a monoclonal anti-immunoglobulin E antibody (omalizumab) for the treatment of allergic respiratory disorders. *Am. J. Respir. Crit. Care Med.* 164(8 Part 2):S6–11
65. Pennington LF, Tarchevskaya S, Brigger D, Sathiyamoorthy K, Graham MT, et al. 2016. Structural basis of omalizumab therapy and omalizumab-mediated IgE exchange. *Nat. Commun.* 7:11610
66. Davies AM, Allan EG, Keeble AH, Delgado J, Cossins BP, et al. 2017. Allosteric mechanism of action of the therapeutic anti-IgE antibody omalizumab. *J. Biol. Chem.* 292(24):9975–87
67. Gasser P, Tarchevskaya SS, Guntern P, Brigger D, Ruppli R, et al. 2020. The mechanistic and functional profile of the therapeutic anti-IgE antibody ligelizumab differs from omalizumab. *Nat. Commun.* 11(1):165
68. Cohen ES, Dobson CL, Käck H, Wang B, Sims DA, et al. 2014. A novel IgE-neutralizing antibody for the treatment of severe uncontrolled asthma. *mAbs* 6(3):756–64
69. Jabs F, Plum M, Laursen NS, Jensen RK, Mølgaard B, et al. 2018. Trapping IgE in a closed conformation by mimicking CD23 binding prevents and disrupts FcεRI interaction. *Nat. Commun.* 9(1):7
70. Drinkwater N, Cossins B, Keeble AH, Wright M, Cain K, et al. 2014. Human immunoglobulin E flexes between acutely bent and extended conformations. *Nat. Struct. Mol. Biol.* 21(4):397–404
71. Chen J-B, Ramadani F, Pang MOY, Beavil RL, Holdom MD, et al. 2018. Structural basis for selective inhibition of immunoglobulin E-receptor interactions by an anti-IgE antibody. *Sci. Rep.* 8(1):11548
72. Stumpp MT, Dawson KM, Binz HK. 2020. Beyond antibodies: the DARPIn® drug platform. *BioDrugs* 34(4):423–33
73. Kim B, Eggel A, Tarchevskaya SS, Vogel M, Prinz H, Jardetzky TS. 2012. Accelerated disassembly of IgE-receptor complexes by a disruptive macromolecular inhibitor. *Nature* 491(7425):613–17
74. Miyajima H, Watanabe N, Ovary Z, Okumura K, Hirano T. 2002. Rat monoclonal anti-murine IgE antibody removes IgE molecules already bound to mast cells or basophilic leukemia cells, resulting in the inhibition of systemic anaphylaxis and passive cutaneous anaphylaxis. *Int. Arch. Allergy Immunol.* 128(1):24–32
75. Meerts P, Cornelis S, Hermans G, Vercammen J, Verdonck FDK. 2011. *Immunoglobulin single variable domains directed against IgE*. WO Patent 2012175740A1
76. Rinaldi M, Denayer T, Thiollay S, Perez Tosar L, Buyse M-A, et al. 2013. ALX-0962, an anti-IgE nanobody with a dual mode of action. *Eur. Respir. J.* 42:1765
77. Eggel A, Baravalle G, Hobi G, Kim B, Buschor P, et al. 2014. Accelerated dissociation of IgE-FcεRI complexes by disruptive inhibitors actively desensitizes allergic effector cells. *J. Allergy Clin. Immunol.* 133(6):1709–19.e8
78. MacGlashan D. 2015. Facilitated dissociation of IgE versus cell replacement. *J. Allergy Clin. Immunol.* 135(1):294–95
79. Pennington LF, Gasser P, Kleinboelting S, Zhang C, Skinotitis G, et al. 2021. Directed evolution of and structural insights into antibody-mediated disruption of a stable receptor-ligand complex. *Nat. Commun.* 12(1):7069

80. Pennington LF, Gasser P, Brigger D, Guntern P, Eggel A, Jardetzky TS. 2021. Structure-guided design of ultrapotent disruptive IgE inhibitors to rapidly terminate acute allergic reactions. *J. Allergy Clin. Immunol.* 148(4):1049–60
81. Paramanathan T, Reeves D, Friedman LJ, Kondev J, Gelles J. 2014. A general mechanism for competitor-induced dissociation of molecular complexes. *Nat. Commun.* 5:5207
82. Abdiche YN, Yeung AY, Ni I, Stone D, Miles A, et al. 2017. Antibodies targeting closely adjacent or minimally overlapping epitopes can displace one another. *PLOS ONE* 12(1):e0169535
83. Gromadski KB, Wieden H-J, Rodnina MV. 2002. Kinetic mechanism of elongation factor Ts-catalyzed nucleotide exchange in elongation factor Tu. *Biochemistry* 41(1):162–69
84. Freistrotter DV, Pavlov MY, MacDougall J, Buckingham RH, Ehrenberg M. 1997. Release factor RF3 in *E. coli* accelerates the dissociation of release factors RF1 and RF2 from the ribosome in a GTP-dependent manner. *EMBO J.* 16(13):4126–33
85. De Meyts P, Whittaker J. 2002. Structural biology of insulin and IGF1 receptors: implications for drug design. *Nat. Rev. Drug Discov.* 1(10):769–83
86. He MM, Smith AS, Oslob JD, Flanagan WM, Braisted AC, et al. 2005. Small-molecule inhibition of TNF- α . *Science* 310(5750):1022–25
87. Chen T-Y, Cheng Y-S, Huang P-S, Chen P. 2018. Facilitated unbinding via multivalency-enabled ternary complexes: new paradigm for protein-DNA interactions. *Acc. Chem. Res.* 51(4):860–68
88. Prinz H, Striessnig J. 1993. Ligand-induced accelerated dissociation of (+)-*cis*-diltiazem from L-type Ca^{2+} channels is simply explained by competition for individual attachment points. *J. Biol. Chem.* 268(25):18580–85
89. Xolair® [package insert]. Dublin, Ireland: Novartis Europharm Ltd.; 2005
90. Cooper A, Dryden DT. 1984. Allostery without conformational change: a plausible model. *Eur. Biophys. J.* 11(2):103–9
91. Cook JP, Henry AJ, McDonnell JM, Owens RJ, Sutton BJ, Gould HJ. 1997. Identification of contact residues in the IgE binding site of human Fc ϵ RI α . *Biochemistry* 36(50):15579–88
92. Henry AJ, Cook JP, McDonnell JM, Mackay GA, Shi J, et al. 1997. Participation of the N-terminal region of C ϵ 3 in the binding of human IgE to its high-affinity receptor Fc ϵ RI. *Biochemistry* 36(50):15568–78
93. Sayers I, Cain SA, Swan JR, Pickett MA, Watt PJ, et al. 1998. Amino acid residues that influence Fc ϵ RI-mediated effector functions of human immunoglobulin E. *Biochemistry* 37(46):16152–64
94. Ortega E, Schweitzer-Stenner R, Pecht I. 1991. Kinetics of ligand binding to the type 1 Fc epsilon receptor on mast cells. *Biochemistry* 30(14):3473–83
95. Kolkhir P, Altrichter S, Munoz M, Hawro T, Maurer M. 2020. New treatments for chronic urticaria. *Ann. Allergy Asthma Immunol.* 124(1):2–12



Contents

Not Dead Yet <i>Betty Diamond</i>	1
Designing Cancer Immunotherapies That Engage T Cells and NK Cells <i>Oleksandr Kyrysyuk and Kai W. Wucherpfennig</i>	17
Reconciling Mouse and Human Immunology at the Altar of Genetics <i>Philippe Gros and Jean-Laurent Casanova</i>	39
RNA Modification in the Immune System <i>Dali Han and Meng Michelle Xu</i>	73
The Immunological Conundrum of Endogenous Retroelements <i>George Kassiotis</i>	99
Natural Killer Cells in the Human Uterine Mucosa <i>Victoria Male and Ashley Moffett</i>	127
Biomaterials-Mediated Engineering of the Immune System <i>Coralie Backlund, Sasan Jalili-Firoozinezhad, Byungji Kim, and Darrell J. Irvine</i>	153
Extremely Differentiated T Cell Subsets Contribute to Tissue Deterioration During Aging <i>Gonzalo Soto-Herederó, Manuel M. Gómez de las Heras, J. Ignacio Escrig-Larena, and María Mittelbrunn</i>	181
Immune-Epithelial Cross Talk in Regeneration and Repair <i>Laure Guenin-Mace, Piotr Konieczny, and Shruti Naik</i>	207
IL-4 and IL-13: Regulators and Effectors of Wound Repair <i>Judith E. Allen</i>	229
IgE, IgE Receptors and Anti-IgE Biologics: Protein Structures and Mechanisms of Action <i>J.M. McDonnell, B. Dhalwal, B.J. Sutton, and H.J. Gould</i>	255
Host Recovery from Respiratory Viral Infection <i>Xiaoqin Wei, Harish Narasimhan, Bibo Zhu, and Jie Sun</i>	277
Structural Mechanisms of NLRP3 Inflammasome Assembly and Activation <i>Jianing Fu and Hao Wu</i>	301

Systems Immunology Approaches to Metabolism <i>Denis A. Mogilenko, Alexey Sergushichev, and Maxim N. Artyomov</i>	317
T Cell Responses to SARS-CoV-2 <i>Alessandro Sette, John Sidney, and Shane Crotty</i>	343
Origin and Heterogeneity of Tissue Myeloid Cells: A Focus on GMP-Derived Monocytes and Neutrophils <i>Lai Guan Ng, Zhaoyuan Liu, Immanuel Kwok, and Florent Ginhoux</i>	375
Immune Mechanisms in Inflammatory Anemia <i>Susan P. Canny, Susana L. Orozco, Natalie K. Thulin, and Jessica A. Hamerman</i>	405
Complement in the Brain: Contributions to Neuroprotection, Neuronal Plasticity, and Neuroinflammation <i>Suzanne S. Boblson and Andrea J. Tenner</i>	431
Effector-Triggered Immunity <i>Brenna C. Remick, Moritz M. Gaidt, and Russell E. Vance</i>	453
TGF- β Regulation of T Cells <i>WanJun Chen</i>	483
Modeling T Cell Fate <i>Rob J. De Boer and Andrew J. Yates</i>	513
Systemic Lupus Erythematosus Pathogenesis: Interferon and Beyond <i>Simone Caielli, Zurong Wan, and Virginia Pascual</i>	533
Interfering with Interferons: A Critical Mechanism for Critical COVID-19 Pneumonia <i>Helen C. Su, Huie Jing, Yu Zhang, Members of the COVID Human Genetic Effort, and Jean-Laurent Casanova</i>	561

Errata

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