HUMAN ANTIBODY–FC RECEPTOR INTERACTIONS ILLUMINATED BY CRYSTAL STRUCTURES

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Immunoglobulins couple the recognition of invading pathogens with the triggering of potent effector mechanisms for pathogen elimination. Different immunoglobulin classes trigger different effector mechanisms through interaction of immunoglobulin Fc regions with specific Fc receptors (FcRs) on immune cells. Here, we review the structural information that is emerging on three human immunoglobulin classes and their FcRs. New insights are provided, including an understanding of the antibody conformational adjustments that are required to bring effector cell and target cell membranes sufficiently close for efficient killing and signal transduction to occur. The results might also open up new possibilities for the design of therapeutic antibodies.

IMMUNOGLOBULIN DOMAINS The essential building blocks of all immunoglobulins, comprising a globular unit of ~110 amino acids with a predominant β-sheet arrangement that is stabilized by an internal disulphide bond. The β-strands of antibody constant domains are labelled ABCDEFG from the amino-terminus.

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Antibodies exist as five different classes (IgG, IgE, IgA, IgM and IgD) with distinct structures, biological activities and distributions in the body (TABLE 1). IgG — the main antibody class in the serum — has a crucial role in protection against invading bacteria and viruses, whereas IgE helps to protect against infection with parasitic helminths and is also responsible for the debilitating symptoms of allergy. IgA — another major serum antibody — arguably has its key role at mucosal surfaces, such as the linings of the lungs and the gastrointestinal tract, where it is the main immunoglobulin in the secretions that bathe these surfaces. IgM, which is restricted mainly to the circulation due to its large size, is also known to protect against bacterial and fungal infection, whereas the function of IgD remains less clear.

Here, we are concerned with the most well-characterized immunoglobulin classes, that is, IgG and IgA, the two most prevalent classes in the body, and IgE, which is a subject of much interest due to its association with allergy (FIG. 1). In humans, IgG exists as four subclasses (IgG1, IgG2, IgG3 and IgG4) and IgA as two (IgA1 and IgA2), in which the heavy chain of each subclass is the product of a different heavy-chain constant (C) region gene.

All antibody — or immunoglobulin — molecules have a similar basic structural unit comprised of two identical heavy chains and two identical light chains, each folded into globular IMMUNOGLOBULIN DOMAINS. The chains are arranged to form two antigen-binding (Fab) regions that are linked to an Fc region (FIG. 1). Despite this similarity, important differences between the modes of action of different immunoglobulin classes are apparent (TABLE 1).

Most immune functions of antibodies depend on their ability to act as flexible adaptor molecules, linking pathogen with appropriate elimination mechanisms. This 'bridging' role entails two types of recognition, each involving contributions from particular antibody domains. The first involves highly specific recognition of the antigen target, and is mediated through the amino-terminal variable domains of the two Fab regions of the antibody. The second involves interaction of the constant domains of the Fc region of the molecule with various effector molecules, including complement and, perhaps most importantly, Fc receptors (FcRs) present on phagocytes and other immune cells. The dual recognition of target and FcR by immunoglobulin molecules has a key role in eliciting effector mechanisms to rid the body of bacteria, viruses

Table 1 | **Human immunoglobulins**

Class (heavy-chain designation)	Subclass	Main molecular form	Polypeptide chains*	Primary location	Complement activation (pathway)
IgG (γ)	IgG1 IgG2 IgG3 IgG4	Monomer	γ2, L2	Serum (~12 mg/ml), tissues	IgG3>IgG1>>IgG2>>IgG4 (classical)
IgA (α)	IgA1 IgA2	Monomer Dimer Secretory	α2, L2 $(α2, L2)_2$, J $(α2, L2)_2$, J, SC	Serum (~3 mg/ml): 90% monomer, 10% dimer Seromucous secretions, milk, colostrum and tears	Yes (mannan-binding lectin)
IgM (μ)		Pentamer	$(μ2, L2)_5$, J	Serum (~1.5 mg/ml)	Yes (classical)
IgE (ε)		Monomer	ε2, L2	Serum (0.05 μg/ml)	No
IgD (δ)		Monomer	δ2, L2	Serum (30 μg/ml)	No

*Numbers of monomer units in polymeric forms are indicated by subscript figures. J, joining chain; L, light chain; SC, secretory component.

and parasites, and is the focus of this article. We look in detail at the IgG, IgE and IgA classes and their respective FcRs, drawing together recent structural information to compare and contrast their different modes of antibody–receptor interaction. We discuss how this emerging structural information adds to present knowledge on the functions of the various antibody and receptor classes, including their roles in host-cell–target-cell interaction and host-cell activation.

Human immunoglobulin structure

Although the X-ray crystal structure of the human IgG1 Fc region was solved more than 20 years ago[1,2], until recently there was no atomic-level structural information available for IgE and IgA. However, our understanding of these antibody classes has now moved forwards rapidly, based on the information recently indicated by the X-ray crystal structures for the Fc regions of human IgE and IgA1 (REFS 3–6) (online TABLE S1). With these new data, one can finally make comparisons between the structures of the IgG, IgE, and IgA Fc regions and begin to relate the differences to function.

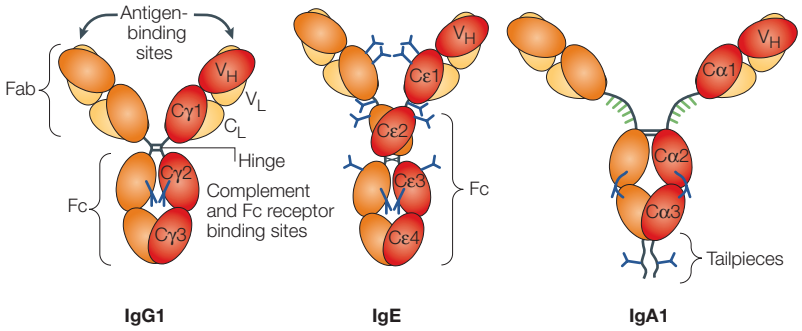

Figure 1 | **The structure of human IgG1, IgE and IgA1.** Immunoglobulins are composed of two identical heavy chains and two identical light chains, linked by disulphide bridges. The class of antibody is determined by its heavy chain, with all classes sharing the same light chains. Light chains fold into a variable domain (V_L) and a constant domain (C_L), whereas heavy chains are composed of one variable domain (V_H) and either three (in IgG and IgA) or four (in IgE) constant domains. The antigen-binding sites at the tip of the Fab regions are formed from the variable domains of both the heavy and light chains. The Fc region mediates interaction with effector molecules, such as complement and Fc receptors. In IgG and IgA, the Fab and Fc regions are separated by a flexible hinge region, which varies in length and sequence between different immmunoglobulin classes and subclasses. In IgE, the classical hinge is replaced by an extra pair of constant domains. For each antibody, one heavy chain is shown in red, the other in orange, with light chains in yellow. N-linked oligosaccharides are shown in blue and O-linked oligosaccharides on the IgA1 hinge region in green. IgA can polymerize through 18 amino-acid carboxy-terminal extensions known as tailpieces.

For IgG, the crystal structure of the Fc region in complex with its receptor differs somewhat from that in the uncomplexed state, with an asymmetrical opening of the structure by ~7 Å between the amino-terminal tips of the Cγ2 domains on receptor binding. The domain arrangement in the IgE Fc region might also adopt different configurations in its receptor-bound and free states[4,5]. For IgA, the lack of a structure for the free IgA Fc region precludes comparisons with that of receptor-bound IgA.

The Fc regions of all three immunoglobulin classes show similarities in overall domain arrangements. Ignoring the extra Cε2 domain pair in IgE, each Fc region is a pseudo two-fold symmetrical dimer of the carboxy-terminal portion of their respective heavy chains (FIG. 2), which is stabilized by interdomain interactions (online TABLE S2). The main differences between the Fc structures are in the positions of the inter-heavy-chain disulphide bridges and the N-linked carbohydrates, and the contributions of carbohydrate to the overall stability of the Fc region (online TABLE S2).

The whole IgE Fc region $(Cε2, Cε3, Cε4)_2$ has been known, since the early 1990s, to adopt a bent conformation both when free in solution and when bound to FcεRI[7,8]. The recent X-ray crystal structure confirmed this configuration, showing that the IgE Fc region is acutely and asymmetrically bent at the flexible linker region between the Cε2 and Cε3 domains[5]. One Cε2 domain folds back against the Cε3 domain of the opposite heavy chain, making additional contacts with the Cε4 domain of this opposite chain, whereas the other Cε2 domain makes only a few contacts with the Cε3 domain of the opposite chain and none with Cε4. The Cε2 domains share a small, mainly hydrophilic interface, unlike other constant-domain pairings. Possibly as a result of the Cε2 domain contacts or due to flexibility in the hinge region between the Cε3 and Cε4 domains, the symmetry of the Cε3 domains is distorted. Whereas one

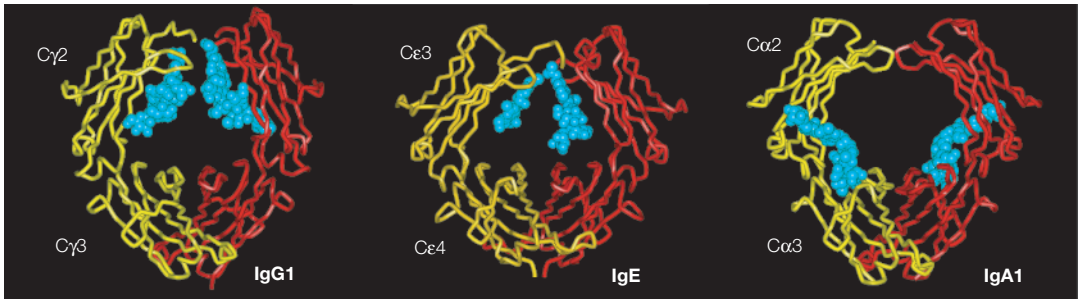

Figure 2 | **Comparison of the structures of the carboxy-terminal domains of the Fc regions of human IgG1, IgE and IgA1.** In each case, the structure adopted by the Fc region when complexed with its cognate Fc receptor is shown. X-ray crystal-structure coordinates were taken from the Protein Data Bank (see further information for website) entries 1IIS (IgG1), 1F6A (IgE) and 1OW0 (IgA1). One heavy chain is shown in red, the other in yellow, and the N-linked oligosaccharides attached to Asn297 in IgG, Asn394 in IgE and Asn263 in IgA are shown in blue. For IgE, the structure does not include the Cε2 domains of the Fc region and shows only the Cε3 and Cε4 domains.

Cε3 domain remains in the 'closed' conformation seen in the uncomplexed structure of the Cε3–Cε4 domains[4], the other approaches the 'open' conformation adopted by both Cε3 domains when IgE binds. This Fc structure might undergo some degree of conformational change on receptor binding, as discussed later.

How do these structures of isolated Fc fragments relate to Fc structure in whole antibody molecules? For IgG, there seems to be a good correlation, as shown by high-resolution crystal structures of intact mouse IgG molecules[9,10] and more recently of human IgG1 with a full-length hinge region[11]. Human IgG1 is clearly capable of marked asymmetry and flexibility, mostly mediated by the hinge region[12], with each Fab region capable of attaining very different positions relative to the Fc region.

For IgE and IgA, there is less information available on whole antibody conformation, restricted mainly to early electron microscope images and models based on solution scattering[7,8,13–17]. Studies based on fluorescence resonance energy transfer between labels attached at either end of the heavy chain have shown that IgE is less flexible than IgG in solution[18], whereas comparisons between antigen-associated antibodies using electron microscopy[16] indicate that the IgA2 subclass might be even more restricted in terms of flexibility, which is unsurprising given the short hinge region of this subclass. The IgA1 subclass was not analysed in this study, but its hinge region is longer and extended[17], and it might be expected to be reasonably flexible.

Human Fc receptors

Specific FcRs have been described for each immunoglobulin class[19–22] (TABLE 2). Most are structurally related, having evolved as members of the immunoglobulin gene superfamily. Each comprises a unique ligand-binding chain (α-chain), which is often complexed through its transmembrane region with a dimer of the common FcR γ-chain. The latter, first isolated as a component of FcεRI but later found to complex with other FcRs, has a key role in the signalling functions of many of the receptors. Fc γ-chains have IMMUNORECEPTOR TYROSINE-BASED ACTIVATION MOTIFS (ITAMs) in their cytoplasmic regions, crucial for the initiation of activatory signals[23]. Some receptor α-chains have their own ITAMs in their cytoplasmic regions, whereas others contain IMMUNORECEPTOR TYROSINE-BASED INHIBITORY MOTIFS (ITIMs) (TABLE 2).

For IgG, three different classes of FcγRs have been characterized, most with several variant forms[24] (TABLE 2). The high-affinity FcγR, FcγRI (CD64), is unusual in that it has three extracellular immunoglobulin-like domains in its ligand-binding chain, whereas all other FcRs have two. FcγRII (CD32) is a medium-affinity receptor that mainly binds to aggregated IgG. FcγRIII (CD16) also binds rather poorly to monomeric IgG, but has low-to-medium affinity for aggregated IgG.

The binding of IgE to its receptor FcεRI is characterized by the markedly high affinity of the interaction, reflecting a slow dissociation rate (half-life of the complex is ~20 hours). FcεRI is a complex comprising a ligand-binding α-chain that is structurally related to those of the FcγRs, a β-chain and the Fc γ-chain dimer. The low-affinity IgE receptor (FcεRII, CD23) is unrelated and is not discussed further.

FcαRI (CD89) is the only well-characterized IgA FcR[22]. Its ligand-binding α-chain is structurally related to those of the FcγRs and FcεRI, but is a more distantly related member of the family. In fact, it has closer homology with members of a family including natural killer cell inhibitory receptors (KIRs), leukocyte immunoglobulin-like receptors (LIRs, also known as LILRs or immunoglobulin-like transcripts, ILTs), and the platelet-specific collagen receptor, glycoprotein VI (GPVI)[25]. These molecules are encoded by a group of genes that are located in the LEUKOCYTE RECEPTOR COMPLEX (LRC) on chromosome 19 (REF. 26).

In physiological terms, it is important that FcRs only initiate elimination mechanisms when they interact with antigen-complexed immunoglobulins[27]. It has long been known that two or more immunoglobulin molecules that are aggregated through interaction with antigen are sufficient to trigger FcR-mediated processes[28]. The precise mechanisms that are triggered vary with immunoglobulin class and the receptor-expression profile of the cells involved, but they

IMMUNORECEPTOR TYROSINE-BASED ACTIVATION MOTIFS (ITAMs). ITAMs consist of two tyrosine-containing Tyr-Xaa-Xaa-Leu boxes interspaced by seven amino acids, crucial for transducing activatory signals. Mutation of either of the tyrosine residues reduces or abrogates signalling. When phosphorylated after receptor crosslinking ITAMs function as sites that promote the activation of cytoplasmic proteins into signalling complexes.

IMMUNORECEPTOR TYROSINE-BASED INHIBITORY MOTIFS (ITIMs). ITIMs, typically Ile/Val-Xaa-Tyr-Xaa-Xaa-Leu/Val, when phosphorylated after receptor crosslinking, function to negatively regulate cytoplasmic signalling complexes.

LEUKOCYTE RECEPTOR COMPLEX (LRC). A group of genes that are located adjacent to each other on human chromosome 19q13.4 encoding a family of proteins including natural killer cell inhibitory receptors (KIRs), leukocyte immunoglobulin-like receptors (LIRs, LILRs or ILTs) and FcαRI.

Table 2 | **Human leukocyte FcγR, FcεR and FcαR**

Fc receptor (FcR)	Major isoforms expressed	Allotype	Specificity for human Ig*	Affinity for monomer Ig	Signalling motif	Cellular distribution	X-ray crystal structure available
FcγRI (CD64)	FcγRIa	–	IgG1=3>4 IgG2 doesn't bind	High	γ-chain ITAM	Monocytes, macrophages, neutrophils (IFN-γ stimulated), eosinophils (IFN-γ stimulated)	No
FcγRII (CD32)	FcγRIIa	LR	IgG3≥1=2 IgG4 doesn't bind	Low	α-chain ITAM	Monocytes, macrophages, neutrophils, platelets and Langerhans cells	Uncomplexed[35,36]
	FcγRIIa	HR	IgG3≥1>>>2 IgG4 doesn't bind	Low	α-chain ITAM	Monocytes, macrophages, neutrophils, platelets and Langerhans cells	Uncomplexed[35,36]
	FcγRIIb	–	IgG3≥1>>2>4	Low	α-chain ITIM	Monocytes, macrophages and B cells	Uncomplexed[37]
	FcγRIIc	–	N.D.	Low	α-chain ITAM	Monocytes, macrophages, neutrophils and B cells	Extracellular region identical to that of FcγRIIb[37]
FcγRIII (CD16)	FcγRIIIa	–	N.D.	Medium	γ-chain ITAM	Macrophages, NK cells, γδ T cells and some monocytes	No
	FcγRIIIb	NA1, NA2	IgG1=3>>>2=4	Low	No signalling motif Anchored in the membrane by GPI linkage	Neutrophils and eosinophils (IFN-γ stimulated)	Uncomplexed[38,39] and complexed with IgG Fc[38,40]
FcεRI	FcεRI	–	IgE	Very high	γ-chain ITAM β-chain also present, but its role is unclear	Mast cells, basophils, Langerhans cells and activated monocytes	Uncomplexed[41,42] and in complex with IgE Cε3-Cε4 (REF. 3)
FcεRII	FcεRIIa	–	IgE	Low	C-type lectin	B cells	No
(CD23)	FcεRIIb	–	IgE	Low	C-type lectin	B cells, T cells, monocytes eosinophils and macrophages	No
FcαRI (CD89)	FcαRIa	–	Serum IgA1=2, SIgA1=SIgA2	Medium	γ-chain ITAM	Neutrophils, monocytes, some macrophages, eosinophils, Kupffer cells and some DCs	Uncomplexed[6,43] and complexed with IgA Fc[6]

*Relative affinities of various ligands for each receptor are indicated in decreasing order, starting with the isotype with the highest affinity. Arrowheads and equal signs are used to show the differences in affinity. Whereas some receptors signal directly through activatory or inhibitory motifs in their ligand binding α-chain, others depend on membrane association with the Fcγ-chain to allow signalling through the γ-chain ITAM. DC, dendritic cell; GPI, glycosylphosphatidylinositol; IFN-γ, interferon-γ; ITAM, immunoreceptor tyrosine-based activation motif; ITIM, immunoreceptor tyrosine-inhibitory motif; HR, high responder; LR, low responder; NA, neutrophil antigen; N.D., not determined; NK, natural killer; SIgA, secretory IgA.

include phagocytosis and antibody-dependent cellular cytotoxicity (ADCC) of antibody-coated targets, and the release (through degranulation or directly) of proteolytic enzymes, potent activated oxygen species or inflammatory mediators. The cellular components of the signalling pathways have been reviewed elsewhere[21,22,29], but essentially for the FcRs that associate with the Fc γ-chain or contain their own ITAM sequence, receptor crosslinking triggers phosphorylation of ITAMs by protein tyrosine kinases[21,30,31], with subsequent steps involving modulation of multimolecular adaptor complexes that function to regulate signalling. FcγRIIb, when co-aggregated with ITAM-containing receptors, functions as a negative regulator of activation.

Adding a further layer of complexity, certain co-signalling molecules associate with FcRs. For example, macrophage receptor 1 (MAC1, also known as complement component receptor 3, CR3 or CD11b/CD18) has been implicated in the function of FcγRs[32]. MAC1 also seems to function as a co-receptor for FcαRI. Mice lack an equivalent of FcαRI, but in mice transgenic for human FcαRI and deficient in MAC1, the capacity for IgA-mediated extracellular lysis is abolished, indicating a role for MAC1 in FcαRI-mediated ADCC[33]. It has also been reported that MAC1 is required for binding of secretory IgA (SIgA) to FcαRI expressed by neutrophils[34].

Human Fc receptor structure

To date, atomic-level structural data are available for FcγRIIa[35,36], FcγRIIb[37], FcγRIIIb[38–40], FcεRI[3,41,42] and FcαRI[6,43] (TABLE 2). In all of these cases, the structures include the two immunoglobulin-like extracellular domains of the receptor α-chain, known as D1 (amino-terminal, membrane distal) and D2 (carboxy-terminal, membrane proximal). No structure is yet available for the cytoplasmic portions of any of the receptors.

The extracellular regions of FcγRIIa/FcγRIIb, FcγRIII and FcεRI share the same overall heart-shaped structure in which the two domains are positioned at an acute angle (~70°) to each other and share a large interface (FIG. 3). The structures of these receptors are so similar that they can be easily superimposed[36]. The D1 and D2 domains of all the receptors are each arranged into an identical overall immunoglobulin-fold composed of a sandwich of two β-sheets, one five-stranded and the other three-stranded. The β-strands are labelled AA′BCC′EFG from the amino-terminus.

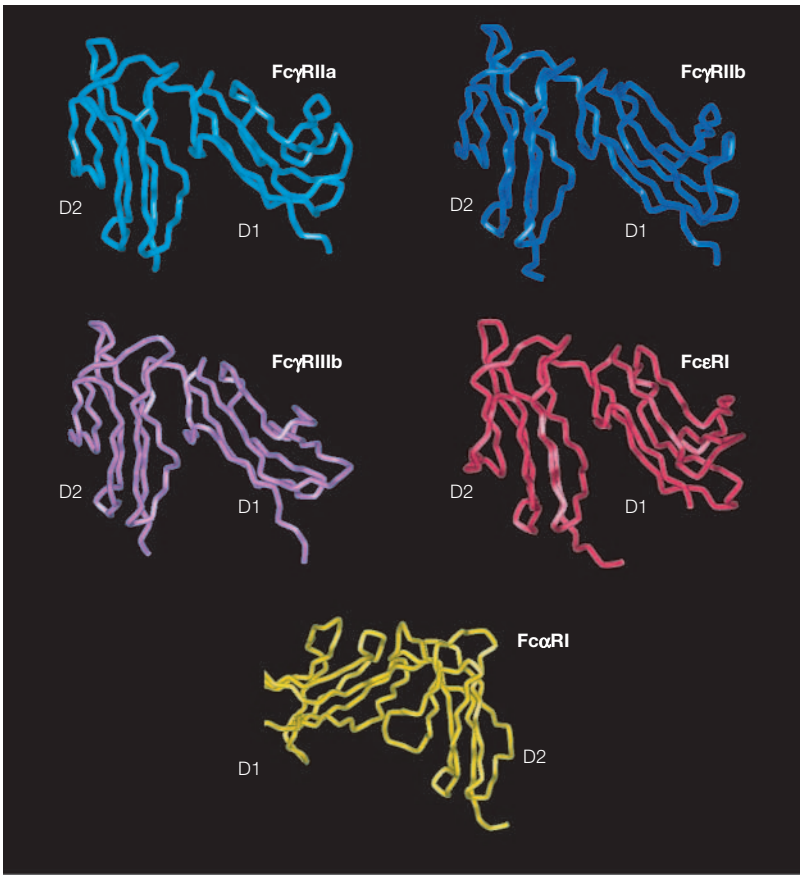

Figure 3 | **Structures of human Fc receptors (FcRs).** A comparison of the X-ray crystal structures of the extracellular domains of human FcγRIIa, FcγRIIb, FcγRIIIb, FcεRI and FcαRI. In each case, a similar view of the receptor is shown, in its uncomplexed state. X-ray crystal-structure coordinates were taken from the Protein Data Bank entries 1FCG (FcγRIIa), 2FCB (FcγRIIb), 1FNL (FcγRIIIb), 1FZQ (FcεRI) and 1OVZ (FcαRI). For the FcγRs and FcεRI, the ligand-binding site is present at the 'top' of the D2 domain. In FcαRI, the D1–D2-domain arrangement is reversed, and the ligand-interaction site is present at the top of the D1 domain. D1, membrane distal; D2, membrane proximal.

Despite the basic sequence similarity between FcαRI and these receptors, the IgA receptor turns out to have a markedly different structure. Whereas the two individual domains of the FcαRI extracellular portion fold in a similar manner to those of the other receptors, the arrangement of the domains relative to each other is different. The domains are rotated by ~180° from the positions adopted in the other FcRs, essentially inverting the D1–D2 orientations. In fact, the structure more closely resembles those of other members of the LRC, particularly KIRs[44] and LIR1[45]. The domains lie at ~90° to each other and have a large hydrophobic interface[6] (FIG. 3). Moderate flexibility at the domain interface might allow the interdomain angle to vary slightly.

The IgG–FcγR interaction

Before the structures of antibody–FcR complexes were solved, mutagenesis studies provided important clues as to the regions of both proteins involved in the interaction. For FcγRI, mapping efforts concentrated on mouse FcγRI[46], whereas both FcγRII[47,48] and FcγRIII[49] were analysed in the human form. A theme emerged, suggesting a crucial role for the membrane proximal D2 domains. Three proximal loops of the FcγRII D2 domain (the FG loop, the B/C loop and the C′/E loop) seemed to have key roles. A corresponding region, along with the CC′ loop, in the D2 domain of FcγRIII was similarly implicated.

Turning to IgG, our own binding analysis of IgG molecules of different subclass and species coupled with sequence alignments provided the first evidence that localizes the interaction site for FcγRI to the amino-terminal region of the Cγ2 domains, within the so-called lower hinge region[50]. The sequence Leu234–Gly237 was highlighted as a crucial region for the interaction. Our mutagenesis studies and those of others supported this site proposal[51–54], indicating that Leu235 has a central role. We also showed that this same region was important for the binding of human FcγRII[52]. For FcγRIII, the picture was less clear at this stage.

More than a decade after the FcR binding sites on IgG were proposed, crystal structures of a complex of the human IgG1 Fc region with the extracellular domains of FcγRIII were described[38,40] (FIG. 4). In the 1:1 complex, the D2 domain of the receptor is docked between the two Cγ2 domains of the Fc fragment. Comparison of the complex structures with those of free receptor and ligand indicates that both molecules undergo some degree of domain rearrangements on binding. The interdomain angle of FcγRIII opens by ~10°, whereas the top of the Cγ2 domains opens slightly, with a concomitant introduction of asymmetry. The D2 domain and residues from the D1–D2 linker of the receptor interact with the Cγ2 domains and the lower hinge regions of IgG, through a combination of salt bridges, hydrogen bonds and hydrophobic interactions. The regions of FcγRIII and IgG involved in the interaction[38,40] are generally consistent with the earlier predictions from mutagenesis studies. The specific regions of FcγRIII involved are Trp110–Ala114 of the B/C loop, Val155–Lys158 of the F/G loop, His116–Thr119 of the C strand and Asp126–His132 of the C′ strand of the D2 domain. Additional contributions are made by Arg152 and Ile85–Trp87 in the D1–D2 linker. These regions contact the lower hinge regions (Leu234–Ser239) on both IgG heavy chains, the first residue of the carbohydrate attached to Asn297, and on one heavy chain Asp265–Glu269 and Asn297–Thr299, and on the other Ala327–Ile332. The receptor components bind the two heavy chains of the Fc region differently, but essentially the contact residues can be grouped into two main IgG–FcR-interaction regions. One comprises a sandwich of Pro329 of one IgG heavy chain between Trp87 and Trp110 of the receptor, and the other involves the interaction of the lower hinge region (Leu234–Ser239) of both IgG heavy chains with different surfaces of the receptor. To interact with the receptor, the hinge peptides of IgG must assume stable asymmetrical conformations, such that their orientations preclude binding of a second receptor molecule by steric hindrance and result in 1:1 stoichiometry.

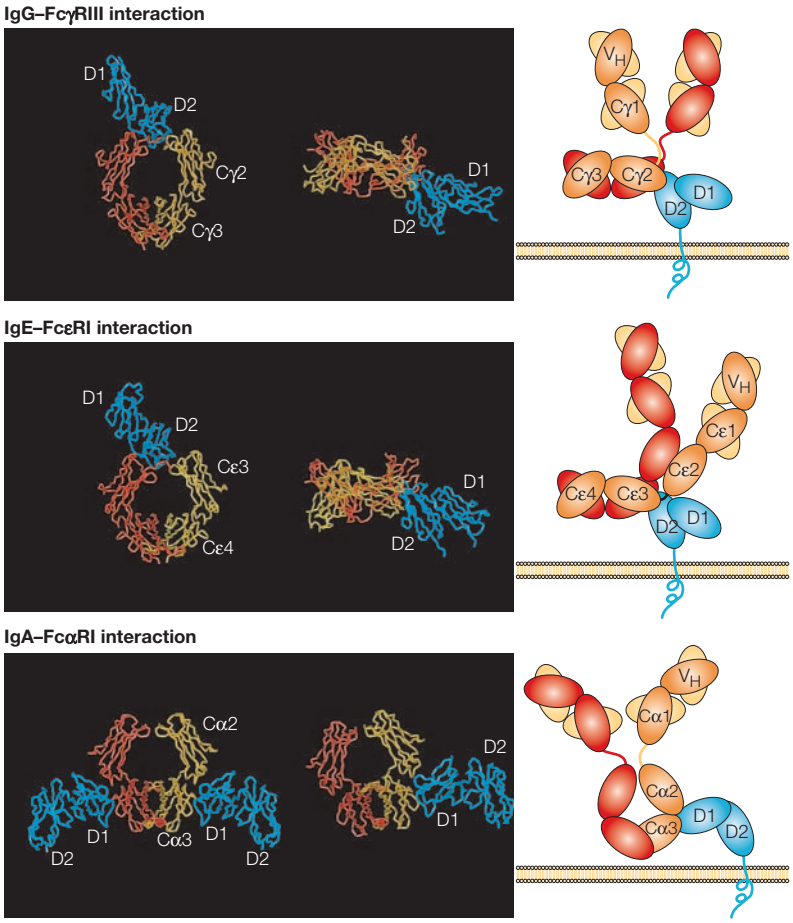

Figure 4 | **Comparison of the IgG–FcγRIII, IgE–FcεRI and IgA–FcαRI complexes.** The left-hand side and middle columns show views of the X-ray crystal structures of the complexes of the receptors with their respective Fc ligands, in which the extracellular domains of the receptors are shown in blue, whereas one heavy chain of each Fc region is shown in red and the other in gold. In the left-hand column, each Fc region is viewed face on. The similarity between the IgG–FcγRIII and IgE–FcεRI interactions is marked, whereas the IgA–FcαRI interaction is quite different in terms of the sites involved and the stoichiometry. The middle column shows a view in which the D2 domains of each of the receptors are positioned so that their carboxy-termini face downwards. Here, the Fc regions of IgG and IgE are seen in a horizontal position from the side. For the IgA interaction, only one receptor molecule is shown. X-ray crystal-structure coordinates were taken from the Protein Data Bank entries 1E4K (IgG Fc–FcγRIII), 1F6A (IgE Fc–FcεRI) and 1OW0 (IgA Fc–FcαRI). The right-hand side column shows a schematic representation of the receptors and their intact ligands from the same viewpoint as the images in the middle column. Light chains are shown in yellow. The requirement for dislocation of IgG and IgE to allow positioning of the antigen-binding (Fab) region away from the receptor-expressing cell surface is apparent.
C, constant domain; D1, membrane distal; D2, membrane proximal.

Given the similarity between all of the three FcγR classes, together with the earlier predictions that the IgG lower hinge region has a key role in the interaction with FcγRI and FcγRII, it seems probable that all of the FcγRs share a common mode of interaction with IgG. Indeed, it has been possible to produce model complexes between IgG Fc and FcγRI and FcγRII based on the FcγRIII–IgG Fc structure and thereby rationalize particular binding characteristics[36]. For example, one of the IgG Leu235 residues is predicted to bind to a more hydrophobic region in FcγRI than in FcγRIII due to replacement of His116 and His132 in FcγRIII with Tyr and Trp, respectively, in FcγRI. This might contribute to the higher overall affinity of IgG for FcγRI.

The IgE–FcεRI interaction

The IgE-binding site on FcεRI was previously mapped to the D2 domain, with the BC, C′E and FG loops being particularly implicated[55]. The binding site for FcεRI on IgE has been well investigated, resulting in several site proposals[56,57]. A view that favours a crucial role for the Cε3 domains with involvement of the Cε2–Cε3 linker region tended to prevail. Remaining uncertainties were resolved by the X-ray crystal structure of the complex between FcεRI and the Cε3–Cε4 fragment of IgE[3]. The structure reveals that interaction with the receptor mainly involves the Cε3 domains and the Cε2–Cε3 linker regions with no direct contribution from the Cε4 domains (FIG. 4). Essentially there are two distinct interaction sites. In the first, a pocket between the BC and FG loops of one of the Cε3 domains and the Cε2–Cε3 linker binds around Tyr131 in the CC′ region of the D2 domain of the receptor. In the second, Pro426 of the other Cε3 domain sandwiches between tryptophan residues Trp87 and Trp110 at the top of the receptor D1–D2 interface. The whole interaction is highly reminiscent of the FcγRIII–IgG Fc complex. Docking of the receptor to the Cε2–Cε3 linker region (the equivalent of the lower hinge region in IgG) induces a structural asymmetry that is similar to that seen in the IgG lower hinge region on FcγRIII binding. It dictates the 1:1 stoichiometry of the interaction, because entry of a second receptor molecule is obstructed and docking prevented.

FcεRI shows little change in conformation on ligand binding. By contrast, it has been proposed that IgE undergoes marked structural rearrangements on receptor ligation. Comparisons between the X-ray crystal structure of the Cε3–Cε4 fragment complexed with FcεRI and that of the whole uncomplexed Fc region (Cε2–Cε4 domains), and nuclear magnetic resonance (NMR) studies inferring Cε2-domain–FcεRI interactions[58], have been interpreted to favour a conformational change involving both the Cε2 and Cε3 domains in a biphasic binding mechanism[5]. Indeed, conformational change in the IgE Fc region on FcεRI binding had been hinted at by earlier circular-dichroism spectroscopy experiments[59] and monoclonal-antibody-reactivity studies[60]. However, it should be noted that the hypothesized specific interaction between the Cε2 domains and FcεRI is weak and the evidence of large conformational changes in the IgE Fc region is far from universally accepted. It seems that the parameters of the IgE–FcεRI interaction can be explained without invoking any large conformational changes in the IgE Fc region. For example, steric effects and interactions within the Fc region might function to stabilize the bound conformation and explain the contribution of the Cε2 domain to the high affinity of IgE–FcεRI binding. Whatever the mechanism, the exceptionally slow dissociation rate of the IgE interaction, together with restricted tissue diffusion that allows rebinding to receptors, accounts for a residence time on tissue-resident mast cells of ~14 days. This long-term association of IgE with the receptor explains the immediacy of symptoms of hypersensitivity seen in allergic patients on re-encountering allergen.

The IgA1–FcαRI interaction

From the earliest localization experiments it has been apparent that the mode of interaction of IgA with its receptor does not conform to the common pattern that is seen with IgG and IgE and their receptors. Through a series of mutagenesis studies, the FcαRI interaction site on IgA was localized to the interface of the Cα2 and Cα3 domains. The implicated residues are present in one loop in the Cα2 domain (Leu257 and Leu258) and in another in the Cα3 domain (Pro440–Phe443)[61,62]. An interaction site on FcαRI was also proposed on the basis of mutagenesis studies, comprising several proximal residues that form a surface on the membrane-distal D1 domain[63–65]. Important residues included Tyr35, Tyr81 and Arg82, with further contributions from Arg52 and Gly84, His85 and Tyr86 (REFS 63,65). The recent crystal structure of the tailpiece-less Fc region of IgA1 complexed with the extracellular domains of FcαRI has confirmed the location of both of these sites[6] (FIG. 4). The FcαRI–IgA1 Fc interface is characterized by a hydrophobic core that is flanked by a region in which interactions between polar groups predominate. Although these peripheral interactions might include hydrogen-bond interactions, no salt bridges are observed — a finding that is consistent with the minor contribution of electrostatic interactions to the free energy of binding predicted by surface plasmon resonance experiments[66] and further mutagenesis studies[67]. In terms of glycan involvement, an FcαRI carbohydrate attached to Asn58 forms potential hydrogen bonds and a van der Waals contact with the IgA1 Fc region, but that attached to Asn263 of the Cα2 domain of IgA1 does not have a role in the interaction, in keeping with earlier functional studies[68].

On the basis of these site localizations, it has been speculated that the intriguing inability of SIgA to trigger phagocytosis[69] might be explained by the binding of secretory component to the IgA Fc region in such a way as to occlude the FcαRI-binding site[6]. However, further clarification is required, as the precise contact points between the secretory component and IgA in SIgA remain unclear, and although SIgA doesn't trigger phagocytosis, it has been shown to elicit neutrophil respiratory bursts through FcαRI[70]. In the latter case, the integrin co-receptor MAC1 might help to overcome the decreased binding capacity of SIgA for FcαRI.

Comparisons of the antibody–FcR interactions

Clearly there are several important differences between the immunoglobulin–FcR interactions discussed here. The sites recognized on IgG and IgE by the corresponding FcRs occupy approximately analogous positions in the amino-terminal regions of the penultimate constant domains, and these two immunoglobulins bind to analogous sites on the membrane proximal domains of their specific FcRs. Moreover, the interaction surfaces include common features and conserved residues that give rise to the same 1:1 receptor:antibody stoichiometry. By contrast, the FcR-interaction site on IgA is at the domain interface between the penultimate and last carboxy-terminal domains of the Fc region, the IgA-binding site on FcαRI is located in the membrane distal domain, and the stoichiometry of the FcR:IgA interaction seems to be 2:1.

The contrast in interaction modes begs the question as to why the IgA Fc interdomain region has been favoured over the lower hinge as an interaction site for FcαRI. In fact, the Cα2–Cα3 interdomain region and its equivalent region in IgG act as binding sites for several structurally dissimilar proteins. The Cγ2–Cγ3 interface is the site of recognition by protein A[2], protein G[71], the neonatal FcR (FcRn)[72] and many rheumatoid factors[73], whereas the Cα2–Cα3 interface is also recognized by the streptococcal IgA-binding proteins Sir22, Arp4 and β-protein[74]. Due to the highly accessible and hydrophobic nature of this interdomain surface, it has been recognized as one of a limited number of regions on the immunoglobulin surface that is particularly suited to protein–protein interaction[75,76]. This interdomain site might have been favoured during evolution as a result of unusual hinge flexibility or hinge/Cα2 structural relationships in IgA. Particular structural arrangements at the top of the Fc region could inhibit efficient access of a receptor to this region, or prevent the antibody dislocation that is thought to be required for bridging between host-cell FcR and target-cell antigen (see later). In addition, the structure adopted by FcαRI, unique among FcRs, might simply be more compatible with the Fc interdomain site.

Another difference between the antibody–receptor interactions involves the contribution made by N-linked carbohydrates in the Fc region. Although they make no input to the IgA–FcαRI interaction, these glycans seem to make minor contributions to interactions with the receptors in the IgG–FcγRIII and IgE–FcεRI interactions (burying ~100 Å^2 and 50 Å^2, respectively)[77]. These observations explain some earlier functional experiments in which IgG that lacks Fc-region carbohydrates showed markedly reduced binding to FcγRs, whereas IgA lacking the equivalent sugars retained normal affinity for FcαRI[19,68]. Indeed, recently, appreciation of the contribution of the N-linked Fc-region carbohydrates of IgG to the interaction with FcγRs has increased. For example, the absence of fucose residues from IgG Fc-region carbohydrates is associated with improved binding to FcγRIII and enhanced ADCC[78,79]. The presence or absence of individual sugar residues influences the overall conformations of the oligosaccharides, which in turn impact on the conformation of the Cγ2 domains. Clearly, the carbohydrate composition of the Fc region can modulate IgG–FcγR interactions[80].

Physiological implications of binding modes

The predicted 2:1 stoichiometry of the FcαRI:IgA interaction merits special consideration. The X-ray crystallographic studies, along with physiochemical experiments[66], indicated that the IgA Fc region can bind two FcαRI molecules simultaneously in solution. However, under normal physiological conditions, it is presumed that crosslinking of two FcR molecules by monomeric IgA does not occur or is insufficient to trigger a signalling cascade. Several possible explanations that are not mutually exclusive

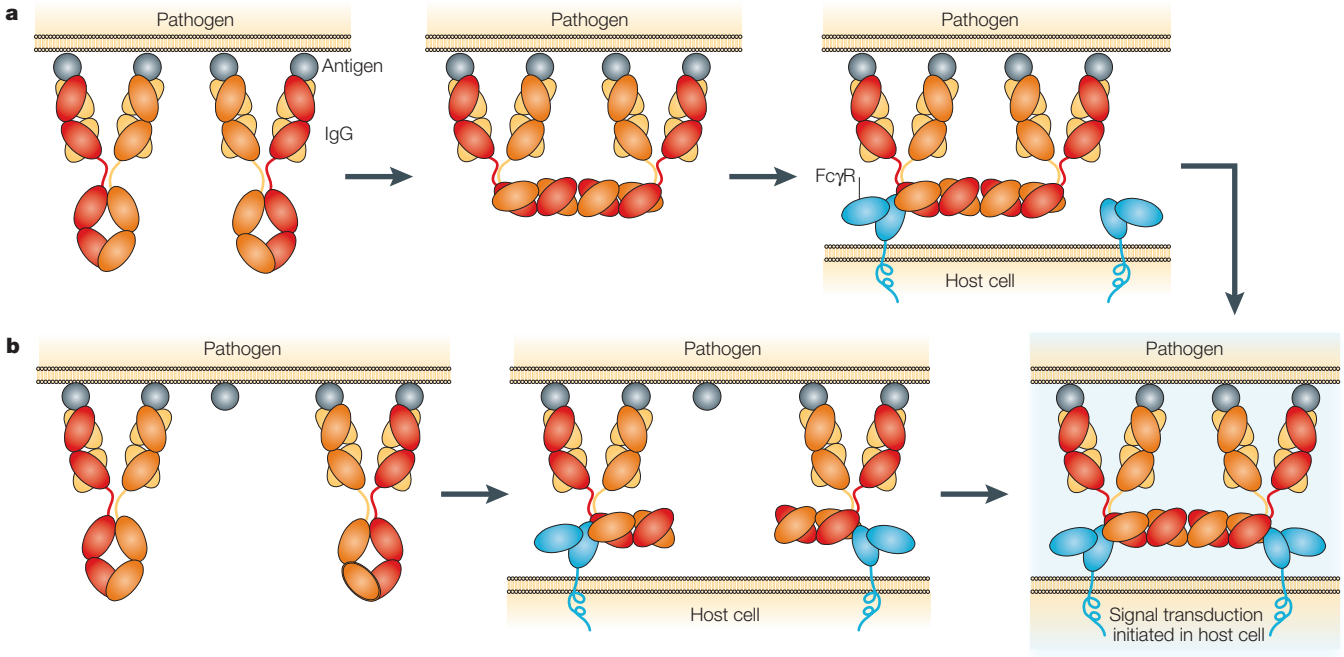

Figure 5 | **Antibody bridging between cells.** A schematic representation showing two IgG molecules bridging between antigen molecules on the surface of a pathogen and the Fcγ receptors (FcγRs) on a host immune cell. For each IgG molecule one heavy chain is red, the other orange and the light chain is yellow. FcγRs are shown in blue. One can envisage various possible ways that antigen-specific IgG might trigger FcγRs in such a system and two possibilities are shown here. **a** | The first is initiated by the binding of IgG molecules to antigen on the pathogen surface. These IgGs dislocate and the formation of arrays stabilizes this conformation, such that on approach of a FcγR-expressing cell, a receptor molecule might bind. After random receptor movement within the host-cell membrane, a second receptor then interacts. Further membrane rearrangements of the host cell occur, including for example, relocation of the FcγRs to membrane rafts, culminating in signal transduction. **b** | In the second way, two IgG molecules bind to the antigen surface at some distance apart. They dislocate, allowing binding of FcγRs. Membrane rearrangements facilitate array formation, which stabilizes the close arrangement of FcγRs with subsequent signalling. This is a simplified representation, which is not meant to imply that either dimeric arrangements or Cγ3–Cγ3 interactions are necessarily favoured. A higher order oligomer arrangement with interactions through Cγ2–Cγ3 interfaces is perhaps more probable.

have been proposed[6]. First, in the crystal structure of the IgA–FcαRI complex, the two receptors bound to IgA are separated by ~125 Å at their carboxy-termini. If two receptors were spaced at such a distance apart in the cell membrane bound by the same IgA molecule, they might be too far apart to trigger efficient signal initiation. Signalling most probably requires close clustering of numerous receptors by IgA-immune complexes. Second, the receptors might be tethered to cytoskeletal elements, which might not be triggered to undergo the required rearrangements that are compatible with receptor clustering below a certain level of receptor ligation or before cell priming by other factors such as cytokines. Third, the high levels of circulating IgA might favour 1:1 complexes, until these are displaced by incoming IgA-immune complexes. Multivalent interactions with larger immune complexes would provide slow off-rates, allowing triggering events to occur.

On a more general theme, many targets for elimination by FcR-mediated effector mechanisms are whole cells, whether bacterial or parasitic invaders or virus-infected cells or, with the increased clinical use of monoclonal antibodies, host cells such as tumour cells. To mediate the eradication of such cells, antibodies must form a bridge between antigen on the target cell and FcRs on the immune cell. Here, various energetic considerations and factors other than simple affinity of the antibody for the FcR come into play[19]. The bringing together of two cell surfaces requires an input of sufficient free energy from stabilizing interactions to overcome the natural forces of repulsion that will tend to oppose the close approach of such surfaces. For optimal bridge formation and subsequent signal transduction, the FcR, antibody and antigen molecules must all be able to assume appropriate positions, conformations and orientations relative to one another.

So what might be the probable impact of the different antibody–FcR binding modes on the relative ability of different immunoglobulin classes to form such bridges? First, the structure of the antibody itself will influence the distances it can span. For example, the extra length of the IgG3 hinge region enables it to mediate ROSETTE FORMATION more efficiently than the shorter hinged IgG1, when all of the other parameters are similar[81]. So, it might be assumed that IgA1, if its extended hinge region is sufficiently flexible, should be particularly efficient at forming intercellular bridges.

Second, the flexibility of the antibody is expected to be crucial for efficient bridge formation. IgG seems

ROSETTE FORMATION
A method of assessing Fc receptor (FcR) interactions, in which the interaction of FcR-expressing cells with erythrocytes coated in erythrocyte-specific antibody is visualized by microscopy. A rosette comprises an FcR-expressing cell bound by three or more erythrocytes. Rosette formation serves as a model of FcR-expressing cell–target-cell interaction

to be highly flexible[12], and IgE, although its Fc region is bent, presumably has flexible movement of the Fab regions relative to the Fc region. IgA1 might be expected to have reasonable flexibility, but the restricted hinge region of IgA2 might compromise Fab movements relative to the Fc region[16].

Third, the actual mode of interaction will affect the ability to form bridges by restricting the range of possible conformations in which the particular antibody can simultaneously bind receptor and antigen. For IgG and IgE, the interaction sites for FcR at the top of the Fc region would seem to dictate that antibody bridging can only be facilitated by some degree of antibody dislocation — that is, movement of the Fc region out of the plane of the Fab arms due to the flexibility of the hinge region[82]. After antigen recognition, antibody dislocation would allow the Fc region to rotate perpendicular to the plane of the Fab arms, enabling interaction with the appropriate FcR. FIG. 5 shows schematic representations of two IgG molecules bridging between antigen molecules on the surface of a pathogen and FcγR on a host immune cell, in which antibody dislocation is required to allow bridging to occur. One might envisage various ways by which antigen-specific IgG could trigger FcγR in such a system and two of these possibilities are shown in FIG. 5, although there is no experimental evidence to distinguish between the different models. IgG has been shown to be capable of adopting such dislocated conformations, and it has been proposed that dislocated IgG molecules might self-associate into arrays, reminiscent of the staple configuration of antigen-complexed IgM[83]. IgE, with its bent configuration, might be predisposed to form similar arrays. Such arrays might function as a potential means to draw together many bridging antibodies so that signalling events might be induced. After antibody-induced crosslinking, FcRs are known to relocate to specialized sphingolipid-cholesterol-rich compartments in the plasma membrane, which are rich in signalling molecules and presumably act as platforms for the recruitment of signalling molecules and the initiation of signal transduction[27,84–89]. One might speculate that array formation could help such relocation into lipid rafts.

For IgA, it is not necessary to incorporate dislocation into any model of a host-cell–target-cell bridge because of the position of the FcαRI-binding site further down the Fc region. However, dislocation is not necessarily precluded, depending on the flexibility of the region that links the D2 domain of the receptor to the cell membrane.

Concluding remarks

Clearly, enhanced knowledge of antibody and FcR structure is shedding new light on how these molecules most probably interact in physiological settings. However, the situation *in vivo* is multifaceted. Antibody responses to any particular pathogen are often polyclonal and involve multiple antibody isotypes. So, the final outcome, in terms of a cellular response mediated by FcRs, will depend on the complex interplay between different FcRs and their ligands. Analysis of FcR ligation by physiologically relevant mixtures of different antibodies might, in the future, help to explain how precise cellular responses are controlled.

We have clear pictures of how individual Fc regions interact with individual FcRs, and, at least for IgG and IgE, antibody dislocation offers a theoretical solution to the steric problems presented by simultaneous antigen and FcR ligation. However, hard experimental evidence for such conformational rearrangements is somewhat lacking. Structural information on large macromolecular complexes is a tall order, but given advances in techniques, such as cryoelectron microscopy, the future might hold possibilities for further elaborating the model.

In recent years, there has been increased interest in the therapeutic use of antibodies, for various applications[90–93]. Detailed knowledge of effector function sites can make a vital contribution, as it allows antibody function to be tailored for particular therapeutic applications. In fact, antibodies with altered effector function have already been shown to be of use in the clinic. For example, IgG molecules with specific mutations that prevent FcγR binding have proved particularly valuable in the treatment of autoimmune diseases and transplant rejection, as they retain the antigen specificity of the antibody but are unable to trigger unwanted immune-cell reactions, such as neutrophil degranulation[94]. As horizons broaden to incorporate new specificities and new isotypes, such as IgA[95], a detailed understanding of immunoglobulin effector function is likely to become increasingly important.

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Competing interests statement
The authors declare that they have no competing financial interests.

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