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► To cite this version:

Bianca Balbino, Eva Conde, Thomas Marichal, Philipp Starkl, Laurent L. Reber. Approaches to target IgE antibodies in allergic diseases. *Pharmacology and Therapeutics*, 2018, 191, pp.50-64. 10.1016/j.pharmthera.2018.05.015 . hal-02022741

HAL Id: hal-02022741

<https://hal.sorbonne-universite.fr/hal-02022741>

Submitted on 18 Feb 2019

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Approaches to target IgE antibodies in allergic diseases

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Abstract

IgE is the antibody isotype found at the lowest concentration in the circulation. However IgE can undeniably play an important role in mediating allergic reactions; best exemplified by the clinical benefits of anti-IgE monoclonal antibody (omalizumab) therapy for some allergic diseases. This review will describe our current understanding of the interactions between IgE and its main receptors FcεRI and CD23 (FcεRII). We will review the known and potential functions of IgE in health and disease: in particular, its detrimental roles in allergic diseases and chronic spontaneous urticaria, and its protective functions in host defense against parasites and venoms. Finally, we will present an overview of the drugs that are in clinical development or have therapeutic potential for IgE-mediated allergic diseases.

Keywords

Immunoglobulin, FcεRI, FcεRII, CD23, omalizumab, DARPins, antibody, allergy, anaphylaxis, asthma, atopic dermatitis

Abbreviations

AD: atopic dermatitis; **Ag**: antigen; **ADAM10**: a disintegrin and metalloprotease 10; **ADCC**: antibody-dependent cell-mediated cytotoxicity; **AECs**: airway epithelial cells; **ASST**: autologous serum skin test; **C ϵ** : constant epsilon domain of IgE; **C_L**: constant region of an antibody's light chains; **CSU**: chronic spontaneous urticaria; **DARPs**: designed ankyrin repeat proteins; **DC**: dendritic cell; **Fab**: fragment antigen-binding region; **Fc**: fragment crystallizable region of an antibody; **HRF**: histamine releasing factor; **IECs**: intestinal epithelial cells; **Ig**: immunoglobulin; **IL**: interleukin; **ITAM**: immunoreceptor tyrosine-based activation motif; **mIgE**: membrane-bound IgE; **PCA**: passive cutaneous anaphylaxis; **PLA2**: phospholipase A2; **PSA**: passive systemic anaphylaxis; **Tg**: transgenic; **T_H2**: T cell helper type 2; **TPO**: Thyroperoxidase; **V_H**: variable region of an antibody's heavy chains; **V_L**: variable region of an antibody's light chains; **WT**: wild type.

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1. Introduction

Immunoglobulin E (IgE) was discovered about 50 years ago. In 1966, the Ishizakas' group in Japan described an immunoglobulin different from the known immunoglobulin classes, that could induce allergic reactions in the skin, and which they called γ E antibody (Ishizaka and Ishizaka 1967). During the same period, the group of Johansson and Bennich in Sweden isolated a new immunoglobulin class, which they called IgND (Johansson and Bennich 1967). It soon turned out that γ E and IgND belong to the same and unique antibody class, and the official name IgE was given in 1968 (Bennich, Ishizaka et al. 1968). The story behind this discovery has been the subject of many reviews, including two recent reviews by the discoverers themselves (Ishizaka and Ishizaka 2016, Johansson 2016). IgE is the isotype found at the lowest concentration in the circulation (50-200 ng/ml IgE in healthy individuals *vs.* \sim 10 mg/ml for IgG) (Dullaers, De Bruyne et al. 2012). However, IgE levels can increase dramatically in individuals with allergic diseases (Galli and Tsai 2012, Platts-Mills, Schuyler et al. 2016). Indeed, the importance of IgE in allergy was demonstrated at the time of its discovery, when the investigators identified that purified IgE was capable of transferring skin reactivity from sensitized human subjects to naive hosts (Ishizaka and Ishizaka 2016, Johansson 2016). This discovery has had great importance for both the diagnosis and treatment of allergic disorders: quantification of allergen-specific IgE is one of the main diagnostic criteria for allergies (Hamilton, MacGlashan et al. 2010), and the anti-IgE therapeutic antibody omalizumab is now approved for the treatment of moderate to severe persistent allergic asthma, and shows great potential for the treatment of other allergic diseases (Humbert, Busse et al. 2014, Pelaia, Vatrella et al. 2015, Kawakami and Blank 2016). Omalizumab has also been approved for the treatment of chronic spontaneous urticaria

(CSU), demonstrating that the pathologic functions of IgE extend beyond allergy (Maurer, Rosen et al. 2013, Chang, Chen et al. 2015, Zhao, Ji et al. 2016).

IgE antibodies exist in two forms: a membrane-bound form (mIgE) expressed by B cells that have undergone class switching to IgE, and a secreted form produced by plasma B cells. mIgE serves as a B cell receptor involved in antigen uptake and presentation. The structure and functions of mIgE, as well as the regulation of IgE synthesis, have been extensively reviewed elsewhere (Geha, Jabara et al. 2003, Gould and Sutton 2008, Wu and Zarrin 2014). This review will focus mainly on the effector functions of secreted IgE (hereafter referred to as 'IgE').

IgE exerts its biological functions by binding to two main receptors: FcεRI and CD23 (FcεRII). The high affinity IgE receptor, FcεRI, is expressed on the surface of blood basophils and tissue resident mast cells; and on other cell types in humans (but not in mice), including neutrophils, eosinophils, platelets, monocytes and dendritic cells (Kraft and Kinet 2007). The low affinity receptor CD23 is expressed mainly by B cells (Sutton and Davies 2015), but also by several other cell populations including neutrophils, eosinophils, follicular DCs and intestinal epithelial cells (IECs) (Acharya, Borland et al. 2010). CD23 on B cells serves mainly as a negative regulator of IgE synthesis (Acharya, Borland et al. 2010). Crosslinking of FcεRI-bound IgE can initiate allergic reactions by inducing the activation of mast cells and basophils, the immediate release of preformed granule-stored mediators such as histamine and proteases, and the *de novo* production of lipid mediators (*e.g.* prostaglandins, leukotrienes), cytokines and chemokines (Galli, Kalesnikoff et al. 2005, Voehringer 2013, Wernersson and Pejler 2014).

145 In this review, we will describe our current understanding of the interactions between
146 IgE and its receptors FcεRI and CD23. We will review the known and potential functions of
147 IgE antibodies in health and disease, in particular their detrimental roles in allergic diseases
148 and chronic spontaneous urticaria, as well as their protective functions in host defense against
149 parasites and venoms. Finally, we will present an overview of the drugs that are in clinical
150 development or have therapeutic potential for IgE-mediated allergic diseases.

2. IgE structure

IgE antibodies are composed of two identical heavy chains (each comprising a variable V_H domain and four constant $C\epsilon$ domains) and two identical light chains (composed of a variable V_L domain and a constant C_L domain) with a total molecular weight of 190 kDa (Gould and Sutton 2008, Wu and Zarrin 2014) (**Figure 1**). Similar to other antibody classes, the Fab region of IgE is responsible for antigen recognition and binding, while the effector function of IgE is determined by the carboxy-terminal Fc portion (Gould and Sutton 2008, Wu and Zarrin 2014). IgE shares a similar overall structure with IgG, with the exception of an additional domain in the heavy chain ($C\epsilon 2$). As detailed in part 3.1.3, this additional $C\epsilon 2$ domain corresponds to the location of the flexible hinge region found in IgG, and plays a major role in enhancing the stability of the interaction between IgE and its high affinity receptor Fc ϵ RI (McDonnell, Calvert et al. 2001). The Fc ϵ RI binding site is located in the $C\epsilon 3$ domain and in the $C\epsilon 2$ - $C\epsilon 3$ linker region (Garman, Wurzburg et al. 2000) (described in more detail in part 3.1.3). The binding site to the low affinity IgE receptor CD23 is also primarily located within the $C\epsilon 3$ domain, with contributions from the $C\epsilon 4$ domain (described in more detail in part 3.2.3) (**Figure 1**). The crystal structure of the human $C\epsilon 3$ - $C\epsilon 4$ domains revealed that, by rotating relatively to $C\epsilon 4$, $C\epsilon 3$ can adopt either ‘open’ or ‘closed’ conformations. This conformational flexibility regulates the binding of IgE to both Fc ϵ RI and CD23 (Garman, Wurzburg et al. 2000, Wurzburg, Garman et al. 2000). These features are discussed in more detail in part 3.1.3 & 3.2.3. Several intra- and inter-domain disulphide bridges control the structure and activity of IgE, which is also regulated by glycosylation at various sites (**Figure 1**). In particular, disruption of the glycosylation site found in the $C\epsilon 3$ domain at asparagine-394 (N394) in humans, and N384 in mouse, abrogates the binding of IgE to Fc ϵ RI,

highlighting the importance of glycosylation modifications in IgE biology (Shade, Platzer et al. 2015).

3. IgE receptors

3.1. The high affinity IgE receptor FcεRI

3.1.1. FcεRI structure and expression

FcεRI is the high affinity receptor for IgE (K_d of $\sim 10^{-9}$ to 10^{-10} M). It is constitutively expressed at high levels on both human and rodent mast cells and basophils as a tetramer formed of one α subunit, one β subunit, and a dimer of disulfide-linked γ subunits (Blank, Ra et al. 1989). The α subunit (FcεRI α) belongs to the immunoglobulin (Ig) superfamily, with an extracellular portion composed of two Ig-like domains (D1 and D2), containing the IgE binding sites, a transmembrane domain and a short cytoplasmic domain which is thought to have no signaling function (Kraft and Kinet 2007) (**Figure 2**). Human FcεRI α is glycosylated at seven sites, and these glycosylations appear to be required for proper interactions with the folding machinery in the endoplasmic reticulum, rather than for binding to IgE (Letourneur, Sechi et al. 1995, Sutton and Davies 2015). FcεRI β has a cytoplasmic immunoreceptor tyrosine-based activation motif (ITAM), which acts as signal amplifier. The FcεRI γ homodimer also contains two ITAM domains, which are responsible for signal transduction (Lin, Cicala et al. 1996, Dombrowicz, Lin et al. 1998).

In humans, but not in rodents, FcεRI is also constitutively expressed as a $\alpha\gamma_2$ trimer at the surface of monocytes (Maurer, Fiebiger et al. 1994, Takenaka, Tanaka et al. 1995),

dendritic cells (DCs) (Maurer, Fiebiger et al. 1996), Langerhans cells (Bieber, de la Salle et al. 1992), neutrophils (Gounni, Lamkhied et al. 2001), eosinophils (Gounni, Lamkhied et al. 1994) and platelets (Joseph, Gounni et al. 1997, Hasegawa, Pawankar et al. 1999). It was reported that expression of the $\alpha\gamma 2$ trimer is increased in peripheral blood monocytes from atopic patients, as compared to healthy controls (Maurer, Fiebiger et al. 1994).

A circulating soluble form of Fc ϵ RI (sFc ϵ RI) of about 40 kDa, and which contains an intact IgE binding site, has been described in human serum (Dehlink, Platzner et al. 2011). However, the cell types that release or shed this protein in humans, and the physiological role of sFc ϵ RI, remain to be identified (reviewed in (Platzner, Ruiter et al. 2011).

3.1.2. Fc ϵ RI functions

Fc ϵ RI plays a key role in mediating the biological functions of IgE *in vivo*, which is best exemplified by the fact that Fc ϵ RI-deficient mice are fully resistant to IgE-mediated passive cutaneous anaphylaxis (PCA) and passive systemic anaphylaxis (PSA) (Dombrowicz, Flamand et al. 1993). These findings are most likely attributable to the $\alpha\beta\gamma 2$ Fc ϵ RI tetramer expressed on the surface of mast cells, since mast cell-deficient mice are also resistant to IgE-mediated PCA and PSA (Miyajima, Dombrowicz et al. 1997, Feyerabend, Weiser et al. 2011, Lilla, Chen et al. 2011). Studies using transgenic mice expressing the human Fc ϵ RI α chain under the control of its own promoter have also given significant insight into the functions of human Fc ϵ RI (Dombrowicz, Brini et al. 1996, Dombrowicz, Lin et al. 1998, Greer, Wu et al. 2014). *hFc ϵ RI α ^{Tg}* mice (bred on a mouse Fc ϵ RI-deficient background) express a ‘humanized’ Fc ϵ RI receptor with a similar cellular distribution as that found in humans (Dombrowicz, Brini et al. 1996, Dombrowicz, Lin et al. 1998, Mancardi, Iannascoli et al. 2008, Greer, Wu et

al. 2014). *hFcεRIα^{Tg}* mice can develop PSA reactions upon sensitization with antigen-specific human or mouse IgE and challenge with the same antigen (Dombrowicz, Brini et al. 1996, Dombrowicz, Lin et al. 1998). Notably, mouse IgE is able to bind both human and mouse FcεRI, while human IgE does not bind the mouse receptor (Conrad, Wingard et al. 1983). PCA reactions can even be induced in *hFcεRIα^{Tg}* mice by intradermal transfer of plasma from allergic patients followed by challenge with the relevant allergen (Zhu, Kepley et al. 2005, Liu, Sun et al. 2013). The αβγ2 tetramer on mast cells is also probably the main trigger of IgE-mediated systemic and cutaneous anaphylaxis in *hFcεRIα^{Tg}* mice, although, to the best of our knowledge, this has not yet been unequivocally demonstrated.

The biological functions of the αγ2 trimer of FcεRI are less well understood. Greer and collaborators recently used *hFcεRIα^{Tg}* mice to demonstrate that internalization of human FcεRI by conventional DCs and monocytes (which express the αγ2 trimer) contributes to serum IgE clearance (Greer, Wu et al. 2014). They injected human IgE into *hFcεRIα^{Tg}* mice and control mice (deficient for both human and mouse FcεRI), and found that serum IgE clearance was markedly accelerated in the transgenic animals. They subsequently demonstrated that human IgE was rapidly endocytosed by conventional DCs and monocytes, and that this endocytosis was associated with the rapid clearance of circulating IgE observed in *hFcεRIα^{Tg}* mice (Greer, Wu et al. 2014). While these findings appear convincing, it remains to be determined the extent to which trapping of circulating IgE by human FcεRI expressed on mast cells also contributes to its clearance. It was recently reported that perivascular mouse mast cells can ‘sample’ circulating IgE directly in the blood by extending cell processes across the vessel wall (Cheng, Hartmann et al. 2013). However, the role of FcεRI in serum IgE clearance seems to be a specific feature of the human receptor, and not

the mouse receptor, as mice deficient in FcεRI clear serum IgE to the same extent as WT mice (Cheng, Wang et al. 2010).

It has also been suggested that human peripheral blood DCs use the αγ2 FcεRI trimer for allergen uptake and presentation to naïve T cells (Maurer, Fiebiger et al. 1996). Using transgenic mice expressing human FcεRIα under the dependency of the CD11c promoter, in an attempt to restrict expression to DCs, these authors found that hFcεRI-expressing DCs can efficiently prime naïve T cells for T_H2 differentiation, and amplify antigen-specific T_H2 responses *in vivo* (Sallmann, Reininger et al. 2011).

3.1.3. Binding of IgE to FcεRI

Mutagenesis studies have helped define the FcεRI binding epitope on IgE. Schwarzbaum and colleagues generated a mutant form of mouse IgE with a deletion of 45 amino acids in the carboxy end of Cε3: this mutant IgE was unable to bind FcεRI (Schwarzbaum, Nissim et al. 1989). Nissim and collaborators produced several chimeric IgE containing the Cε2, Cε3 and Cε4 domains of human IgE (hereafter named Cε2-4), in which various domains were replaced by their murine counterparts. This work confirmed that the FcεRI binding site mapped to the Cε3 domain of IgE (Nissim, Jouvin et al. 1991). In 2000, Garman *et al.* determined the crystal structure of the IgE Cε3-4 dimer bound to the extracellular part of FcεRIα (Garman, Wurzburg et al. 2000). Analysis of this crystal structure confirmed that each of the two chains of the IgE Cε3-4 dimer could bind the receptor using surface loops in Cε3, and revealed contributions of the Cε2-Cε3 linker region (Garman, Wurzburg et al. 2000).

Analysis of the crystal structures of the extracellular portion of human FcεRIα alone (Garman, Kinet *et al.* 1998) or in complex with a dimeric Cε3-4 fragment (Garman, Wurzburg *et al.* 2000) have also provided invaluable insight into how IgE interacts with FcεRI. The extracellular part of FcεRIα is formed of two immunoglobulin domains of about 85 amino acids each (D1 and D2), with a heavily bent D1-D2 interface forming an overall structure of an inverted V shape (Garman, Kinet *et al.* 1998, Garman, Kinet *et al.* 1999) (**Figure 2**). The two Cε3 domains of IgE bind distinct sites on FcεRIα, one site found in the D2 domain, and a second site formed by a cluster of four surface-exposed tryptophans in the D1-D2 interface (Garman, Wurzburg *et al.* 2000). The presence of these two binding sites explains the 1:1 stoichiometry of the IgE-FcεRIα complex, which is essential to ensure that receptor crosslinking and activation occurs only upon multivalent antigen binding to IgE (Garman, Wurzburg *et al.* 2000).

A unique feature of the FcεRI receptor, as compared to other Fc receptors, is the distinctly slow dissociation rate of the IgE-FcεRIα complex ($k_{\text{off}} \approx 10^{-5} \text{ s}^{-1}$). This translates into a half-life of about two weeks for IgE bound to FcεRI (compared to only hours for IgG complexes bound to Fcγ receptors), and ensures that tissue mast cells and basophils remain saturated with IgE (Geha, Helm *et al.* 1985, McDonnell, Calvert *et al.* 2001). McDonnell and collaborators showed that full human IgE molecules and dimeric IgE fragments comprising the Cε2, Cε3 and Cε4 domains (Cε2-4) have identical kinetics of dissociation with FcεRIα, while Cε3-4 displays a markedly enhanced dissociation kinetic (~20-fold), indicating that Cε2 plays a major role in enhancing the stability of the IgE-FcεRIα complex (McDonnell, Calvert *et al.* 2001). More recently, Holdom *et al.* published the crystal structure of human Cε2-4 bound to the extracellular domain of FcεRIα, and confirmed that the Cε2 domain

contributes to the slow dissociation rate of IgE-FcεRIα complexes through conformational changes rather than direct interactions with the receptor (Holdom, Davies et al. 2011).

Analysis of the crystal structures of free vs. receptor-bound IgE Fc domains have revealed that the Cε3 domains of IgE undergo a large conformational rearrangement upon binding to FcεRI (Wurzberg, Garman et al. 2000, Wan, Beavil et al. 2002, Wurzberg and Jardetzky 2009, Holdom, Davies et al. 2011). The free IgE Fc portion was observed in a ‘closed’ conformation in which the FcεRIα binding site in Cε3 is masked (Wurzberg, Garman et al. 2000, Wan, Beavil et al. 2002, Wurzberg and Jardetzky 2009). This masking is achieved as the Cε2 domains in the free Fc fragment are folded back asymmetrically onto the Cε3 and Cε4 domains, locking the Cε3 domains in a ‘closed’ conformation (Wan, Beavil et al. 2002) (**Figure 3**). The authors suggest that free ‘bent’ IgE may first engage FcεRI through only one Cε3 domain, followed by an important conformational change involving Cε2, whereby Cε3 would adopt an ‘open’ conformation, leading to engagement of the second Cε3.

3.2. The low affinity IgE receptor CD23 (FcεRII)

3.2.1. CD23 structure and expression

CD23, also known as FcεRII, is the low affinity receptor for IgE ($K_d = 10^{-5}$ M) (Wurzberg, Tarchevskaya et al. 2006). The structure of CD23 and its interaction with IgE have been reviewed in detail (Sutton and Davies 2015). CD23 self-associates as trimer, and is composed of an IgE-binding ‘head domain’ (which belongs to the C-type lectin superfamily) linked to the membrane by an extracellular coiled-coil stalk region, and a small cytoplasmic N-terminal domain (**Figure 4**). CD23 exists in a membrane-bound form of 45 kDa (mCD23),

as well as in soluble forms of various sizes (sCD23) which are released by proteolytic cleavage at several sites in the stalk region (Sutton and Davies 2015). ADAM10 (‘a disintegrin and metalloprotease 10’) is considered to be the main endogenous protease responsible for cleavage and generation of sCD23 (Weskamp, Ford et al. 2006, Lemieux, Blumenkron et al. 2007). The exogenous house dust mite cysteine protease *Der p 1* is also able to cleave mCD23 at two sites (Schulz, Sutton et al. 1997). mCD23 (hereafter referred to as CD23) is expressed by B cells (Sutton and Davies 2015), and several other cell populations including neutrophils (Yamaoka, Arock et al. 1996), eosinophils (Capron, Truong et al. 1992), follicular DCs (Johnson, Hardie et al. 1986) and IECs (Yang, Berin et al. 2000, Yu, Montagnac et al. 2003). Human CD23 exists as two isoforms (CD23a and CD23b), which differ in the first seven (CD23a) or six (CD23b) amino-acid residues of the cytoplasmic N-terminal part (Yokota, Yukawa et al. 1992, Sutton and Davies 2015).

3.2.2. CD23 functions

CD23 is expressed on the surface of B cells, where it serves as a negative regulator of IgE synthesis. Several publications show increased levels of IgE in mice deficient for CD23 (Stief, Texido et al. 1994, Yu, Kosco-Vilbois et al. 1994, Haczku, Takeda et al. 2000, Riffon-Vasquez, Spina et al. 2000, Lewis, Rapsomaniki et al. 2004). Conversely, transgenic mice overexpressing CD23 in B (and T) cells have markedly reduced levels of circulating IgE after immunization (Payet, Woodward et al. 1999). The regulation of IgE production seems to require the oligomerization of CD23, since serum IgE levels are also increased in mice treated with an antibody that binds to the stalk region of CD23 and thus blocks receptor oligomerization (Kilmon, Ghirlando et al. 2001, Ford, Kilmon et al. 2006). It is possible that CD23 on B cells plays an additional role(s) in regulating serum IgE levels, independently of its effects on IgE production. This was suggested by a study showing that exogenous IgE

349 injected into mice deficient for B cells or treated with an anti-CD23 antibody can be detected
350 in the blood one hour later at levels two-fold higher than in the corresponding control mice
351 (Cheng, Wang et al. 2010). The mechanism through which CD23 regulates serum IgE levels
352 is still unclear, and appears to be independent on B cells, since the administered IgE had
353 similar rates of clearance in B cell-deficient and -sufficient mice (Cheng, Wang et al. 2010).

354
355 In B cells, CD23 has also been implicated in IgE-dependent antigen uptake and
356 presentation to T cells. *In vitro* experiments showed that mouse and human B cells incubated
357 with antigen-specific IgE were up to 100-fold more efficient than untreated B cells at
358 presenting low concentrations of the respective antigen, and this phenomenon was markedly
359 reduced by a CD23 blocking antibody (Kehry and Yamashita 1989, Pirron, Schlunck et al.
360 1990).

361
362 CD23 is expressed on IECs, and such expression is enhanced upon antigen
363 sensitization in rodents (Yang, Berin et al. 2000, Yu, Yang et al. 2001), or exposure to the
364 T_H2 cytokine IL-4 in humans (Tu, Salim et al. 2005). Studies using CD23 blocking antibodies
365 or mice deficient for CD23 have demonstrated that CD23 in IECs is involved in the
366 transepithelial transport of IgE and IgE/antigen complexes into the intestinal lumen (Yang,
367 Berin et al. 2000, Yu, Yang et al. 2001, Tu, Salim et al. 2005). This phenomenon is
368 potentially important for food allergy, since it could explain how IgE and allergens are
369 delivered to mast cells located in the lamina propria beneath the epithelial lining of the gut
370 (Tu, Salim et al. 2005, Gould and Sutton 2008). Similarly, CD23 is expressed on human
371 airway epithelial cells (AECs), where it is also subject to upregulation by IL-4, and *ex vivo*
372 experiments suggest that CD23 in AECs is involved in transepithelial transport of IgE and
373 IgE/antigen immune complexes (Palaniyandi, Tomei et al. 2011). A more recent study using

CD23-deficient mice confirmed that CD23 expressed by AECs is involved in IgE and IgE/antigen transport, and showed that expression of CD23 in lung structural cells is important for the development of allergic airway inflammation (Palaniyandi, Liu et al. 2015).

The soluble form of CD23 (sCD23) can also regulate IgE synthesis. sCD23 exists in several isoforms of different sizes. All isoforms can interact with IgE, but the shorter sCD23 remains monomeric while the longer isoforms associate in trimers (reviewed in detail in (Platzer, Ruiter et al. 2011)). sCD23 isomers can have divergent effects on B cells. Trimeric sCD23 can upregulate IgE synthesis through the co-ligation of CD21 and membrane IgE on B cells (Aubry, Pochon et al. 1992, Hibbert, Teriete et al. 2005, McCloskey, Hunt et al. 2007, Cooper, Hobson et al. 2012), whereas monomeric sCD23 inhibits IgE synthesis in human B cells (McCloskey, Hunt et al. 2007).

3.2.3. Binding of IgE to CD23

Early mutagenesis studies mapped the IgE binding site of CD23 to discontinuous epitopes between residues 160-287 in the C-terminal head domain (Bettler, Maier et al. 1989, Bettler, Texido et al. 1992). These mutagenesis studies also suggested that binding of IgE requires six out of eight extracellular cysteine residues of CD23, which are likely involved in the formation of intramolecular disulfide bridges (Bettler, Texido et al. 1992). The head domain of CD23 is involved in IgE binding, since its proteolytic cleavage by the house dust mite protease *Der p 1* abrogates binding (Schulz, Sutton et al. 1997). Nevertheless, one mutagenesis study suggested that the stalk region of CD23 is also involved in IgE binding (Chen, Ma et al. 2002); a finding that was recently confirmed, indicating that the IgE-CD23 interaction is more complex than previously anticipated (Selb, Eckl-Dorna et al. 2016).

Interestingly, the latter study also demonstrated that mutation of the N-glycosylation site of CD23 (N63) alone is sufficient to enhance binding of IgE (Selb, Eckl-Dorna et al. 2016).

Vercelli *et al.* first demonstrated, using a bank of peptides spanning the IgE C ϵ 2-4 domains, that CD23 recognizes a motif in the C ϵ 3 domain of IgE (Vercelli, Helm et al. 1989). This was confirmed in a study using chimeric IgE molecules in which the human C ϵ 3 domain was replaced by mouse C ϵ 3: these chimeric molecules bound to mouse CD23 and concomitantly lost their ability to bind the human receptor (Nissim, Schwarzbaum et al. 1993). Thereafter, the CD23 binding site on IgE was more precisely mapped to the A-B loop of the C ϵ 3 domain (residues 341-356), with a key role for lysine 352 (Sayers, Housden et al. 2004). More recently, the crystal structure of the soluble head domain of CD23 bound to a C ϵ 3-4 IgE dimer was resolved by Dhaliwal and collaborators (Dhaliwal, Yuan et al. 2012). These authors found that one CD23 molecule binds to each IgE heavy chain, principally via the C ϵ 3 domains but with a contribution from C ϵ 4 (Dhaliwal, Yuan et al. 2012) (**Figure 4**). Although the binding sites for Fc ϵ RI and CD23 are at opposite ends of the C ϵ 3 domain, binding of the two receptors to IgE is mutually exclusive. Indeed, binding of IgE to CD23 induces conformational changes in C ϵ 3, leading to a highly ‘closed’ conformation incompatible with Fc ϵ RI binding (Borthakur, Hibbert et al. 2012, Dhaliwal, Yuan et al. 2012). Similarly, the ‘opened’ conformation adopted by C ϵ 3 upon binding to Fc ϵ RI is incompatible with CD23 binding (Borthakur, Hibbert et al. 2012, Dhaliwal, Yuan et al. 2012) (**Figure 3**). Finally, the crystal structure of CD23 bound to a complete IgE Fc fragment was reported, revealing that the IgE C ϵ 2 domain also contributes to CD23 binding, in addition to the known contributions of the C ϵ 3 and C ϵ 4 domains (Dhaliwal, Pang et al. 2017).

3.3. Other IgE or Fc ϵ RI binding molecules

Mast cells and basophils can be activated by the cytokine-like protein histamine-releasing factor (HRF) (reviewed in (Kawakami, Kashiwakura et al. 2014)). It was shown that HRF could bind to a subset of IgE antibodies via their Fab regions, thereby inducing antigen-independent cross-linking of FcεRI-bound IgE molecules, and that this process could amplify inflammation in mouse models of cutaneous anaphylaxis or allergic airway inflammation (Kashiwakura, Ando et al. 2012). Similarly, the protein Galectin-3 (formerly known as ε binding molecule), which is released by several cell types, can bind to both IgE and FcεRI and induce mast cell and basophil activation via antigen-independent crosslinking of FcεRI (Frigeri, Zuberi et al. 1993, Zuberi, Frigeri et al. 1994). Galectin-3 is also directly produced by mast cells (it is found in the cytoplasm and nucleus of mast cells (Craig, Krishnaswamy et al. 1995)), and it was shown that mast cells derived from the bone marrow of galectin-3 deficient mice displayed reduced activation by IgE and antigen *in vitro* as compared to WT mast cells (Chen, Sharma et al. 2006).

Takizawa and collaborators reported that IgE immune complexes can bind to the mouse IgG receptors FcγRIIB and FcγRIII expressed on mast cells and macrophages, with an affinity similar to that of IgG immune complexes (Takizawa, Adamczewski et al. 1992). They further demonstrated that such binding to FcγRs can induce mast cell activation independently of FcεRI (Takizawa, Adamczewski et al. 1992). IgE immune complexes were also found to bind and activate mouse FcγRIV, expressed on monocytes, macrophages and neutrophils (Hirano, Davis et al. 2007, Mancardi, Iannascoli et al. 2008). Confirming that FcγRIV can act as a low-affinity receptor for mouse IgE, treatment of mice with an anti-FcγRIV antibody inhibited late phase reactions in a model of IgE-mediated passive cutaneous allergic inflammation (Hirano, Davis et al. 2007). In addition, experiments performed in mice

deficient for FcεRI, CD23 and all FcγRs except FcγRIV suggested that the *in vivo* engagement of FcγRIV by IgE immune complexes can synergize with mediators released by IgE-activated mast cells to induce lung inflammation (Mancardi, Iannascoli et al. 2008).

4. Roles of IgE in health and disease

4.1. Pathologic roles of IgE

4.1.1. Immediate hypersensitivity reactions

IgE antibodies are probably best known for their critical role in acute allergic reactions. In allergic individuals, mast cells and basophils have antigen-specific IgE bound to FcεRI expressed on the cell surface (Galli and Tsai 2012). Antigen-mediated IgE/FcεRI crosslinking initiates a complex signaling cascade (Reber and Frossard 2014, Sibilano, Frossi et al. 2014), leading to the eventual activation of these effector cells and the immediate and rapid release of preformed granule-stored mediators (Wernersson and Pejler 2014) (*e.g.*, histamine, serotonin, proteoglycans, proteases and cytokines) and *de novo* production and release of an impressive range of lipid mediators (*e.g.*, prostaglandins, leukotrienes), cytokines and chemokines (Galli, Kalesnikoff et al. 2005, Voehringer 2013). These mediators can act locally or systemically, leading to the clinical features of immediate hypersensitivity, such as bronchoconstriction, urticaria, diarrhea (when acting locally in the airways, the skin and the gut, respectively) (**Figure 5**).

4.1.2. Anaphylaxis

Anaphylaxis is the most extreme manifestation of an allergic reaction. In humans, anaphylaxis can be attributed to an IgE- and mast cell-dependent immediate hypersensitivity reaction in individuals previously sensitized to that allergen (Lieberman, Camargo et al. 2006, Burton and Oettgen 2011, Galli and Tsai 2012). Indeed, quantification of specific IgE levels is used to identify potential triggers of anaphylaxis in patients with a personal history of anaphylaxis (Hamilton, MacGlashan et al. 2010). IgE-dependent anaphylactic reactions can also be recapitulated in mice, in which a local or systemic injection of antigen one day after passive injection of antigen-specific IgE induces features of anaphylaxis (Wershil, Mekori et al. 1987, Dombrowicz, Flamand et al. 1993, Oka, Kalesnikoff et al. 2012).

IgE-mediated anaphylaxis is abrogated in mice lacking the high affinity IgE receptor FcεRI (Dombrowicz, Flamand et al. 1993), as well as in mast cell-deficient mice (Feyerabend, Weiser et al. 2011, Lilla, Chen et al. 2011, Oka, Kalesnikoff et al. 2012), highlighting the importance of IgE-mediated mast cell activation in this reaction. Mast cells likely also play a key role in human anaphylaxis. Indeed, elevated levels of the mast cell specific protease tryptase have been detected during anaphylactic reactions in humans (Schwartz, Metcalfe et al. 1987, Schwartz 2006, Brown, Stone et al. 2013). Moreover, an increased incidence of anaphylaxis was reported in patients with mastocytosis, a disease characterized by increased numbers of mast cells (Schuch and Brockow 2017). By contrast, the role of basophils in anaphylaxis is more debated. So-called “Basophil activation tests” are used to confirm allergen sensitization in human patients. In these tests, which are performed on blood samples *ex vivo*, IgE-mediated activation of basophils is monitored by measuring up-regulation of surface markers such as CD63 and CD203c (Santos, Du Toit et al. 2015, Kim, Kim et al. 2016, Giavina-Bianchi, Galvao et al. 2017). Recently, Korosec and colleagues also reported an increase of CD63 expression on circulating basophils, as well as a

marked reduction in the absolute number of circulating basophils, during anaphylactic reactions to *Hymenoptera venom* in humans (Korosec, Turner et al. 2017). While these data suggest that basophils are activated in human anaphylaxis, they do not however demonstrate a significant contribution to anaphylaxis pathophysiology. Even in mice, the role of basophils in IgE-mediated anaphylaxis remains contentious. Different reports indicate that depletion of basophils does not reduce IgE-mediated local or systemic passive anaphylaxis (Wada, Ishiwata et al. 2010, Sawaguchi, Tanaka et al. 2012). Mukai and colleagues reported that intravenous injection of antigen-specific IgE in mice, followed one day later by subcutaneous challenge with the antigen, can induce a triphasic response (Mukai, Matsuoka et al. 2005). The ‘immediate’ and ‘late-phase’ (6 to 10 h after challenge) responses were dependent on mast cells. However, the third-phase, beginning one to two days after challenge, was independent of mast cells and was abrogated upon depletion of basophils (Mukai, Matsuoka et al. 2005, Obata, Mukai et al. 2007, Sawaguchi, Tanaka et al. 2012). This third-phase delayed response was also absent in mice lacking FcR γ (a signaling subunit shared by Fc ϵ RI and activating IgG Fc γ receptors), and was restored upon engraftment of these mice with basophils purified from WT mice but not from FcR γ ^{-/-} mice (Mukai, Matsuoka et al. 2005). Since this passive model relies on specific IgE antibodies, and not on IgG, these results strongly suggest that direct activation of basophils through Fc ϵ RI is responsible for the delayed allergic skin inflammation observed this model. Using a similar model of IgE-mediated chronic allergic inflammation, Cheng *et al.* also reported markedly reduced eosinophilic dermatitis in basophil-deficient mice as compared to control mice three days after cutaneous challenge with the relevant antigen (Cheng, Sullivan et al. 2015).

The presence of allergen-specific IgE alone does not explain an individual’s susceptibility to allergy and anaphylaxis. Allergen-specific IgE can be detected in subjects

who do not develop clinical symptoms when exposed to the corresponding allergen (Sicherer and Sampson 2010). Conversely, some patients can experience near fatal anaphylaxis despite having low or undetectable levels of circulating allergen-specific IgE (Simons, Frew et al. 2007), which suggests (but does not prove) the existence of IgE-independent pathways of anaphylaxis in humans (recently reviewed in (Finkelman, Khodoun et al. 2016) and (Reber, Hernandez et al. 2017)). More definitive evidence for IgE-independent pathways of anaphylaxis has been obtained using mouse models of active systemic anaphylaxis (ASA), in which mice are sensitized with an antigen (to produce antigen-specific antibodies) and re-exposed later on to the same antigen to induce anaphylaxis (Finkelman, Khodoun et al. 2016, Munoz-Cano, Picado et al. 2016). Mice deficient for IgE or for FcεRI can still partially (Sun, Arias et al. 2007, Arias, Chu et al. 2011, Balbino, Sibilano et al. 2017) or fully (Oettgen, Martin et al. 1994, Dombrowicz, Flamand et al. 1997, Jonsson, Mancardi et al. 2011) develop features of anaphylaxis in these ASA models. Other studies have subsequently shown that mouse IgG antibodies can trigger anaphylaxis in ASA models, through activation of IgG receptors (FcγRs) on the surface of various myeloid cells, including basophils, macrophages and neutrophils (Miyajima, Dombrowicz et al. 1997, Jonsson, Mancardi et al. 2011, Khodoun, Kucuk et al. 2013, Finkelman, Khodoun et al. 2016, Balbino, Sibilano et al. 2017).

4.1.3. Allergic asthma

Asthma is a chronic inflammatory disease of the airways with continual increasing prevalence (Busse and Lemanske 2001, Subbarao, Mandhane et al. 2009). In many patients, the asthmatic condition is associated with allergic reactivity to environmental allergens and elevated levels of IgE antibodies (Busse and Lemanske 2001). In these allergic patients, IgE is thought to contribute to the asthmatic manifestations (Galli and Tsai 2012). Following antigen

exposure in the airways, rapid local IgE/FcεRI-dependent mast cell activation and the immediate hypersensitivity reaction can lead to increased vascular permeability, bronchoconstriction and increased mucus production. A large array of cytokines, growth factors and chemokines secreted by activated mast cells can influence airway remodeling (Galli, Tsai et al. 2008, Moiseeva and Bradding 2011). Finally, IgE can also act on other cell types that express FcεRI or CD23, such as DCs, B cells, basophils or (in humans) eosinophils, which may potentially affect several biological responses associated with the asthmatic response (Galli, Tsai et al. 2008, Galli and Tsai 2012). Supporting the important role of IgE in asthma, the anti-IgE antibody omalizumab has been shown to reduce asthma symptoms in several clinical trials involving patients with moderate-to-severe and severe allergic asthma (reviewed in (Humbert, Busse et al. 2014)) (for more detail see part 5.1, below).

4.1.4. Atopic dermatitis

Eczema, or atopic dermatitis (AD), is a pruritic inflammatory skin disease with dramatically increased incidence over the last decades (Bieber 2008, Dharmage, Lowe et al. 2014). AD manifestations are characterized by pruritus (itching), skin inflammatory lesions associated with cellular infiltration and histopathological changes, and atopy. Indeed, the majority of AD patients exhibit increased serum levels of total and antigen-specific IgE (Leung and Bieber 2003, Laske and Niggemann 2004, Oyoshi, He et al. 2009). The function of IgE in development of AD is supported by the beneficial effect of anti-IgE therapy in a number of clinical studies (Belloni, Andres et al. 2008, Liu, Goodarzi et al. 2011).

Abboud, Staumont-Sallé *et al.* used a mouse model of AD induced by repeated epicutaneous sensitizations with ovalbumin. They reported that several features of this model

(including T_H1 and T_H2 skin responses, mast cell recruitment into draining lymph nodes and IgE production) were reduced in *FcεRI*^{-/-} mice. In this model, T_H2 skin response as well as T cell proliferation and IgG1 production were also reduced in mice lacking the IgG receptor FcγRIII (Abboud, Staumont-Salle et al. 2009). In addition, symptoms of AD were completely absent in mice deficient for FcRγ, a subunit shared by FcεRI and FcγRIII (and several other FcR). The authors therefore concluded that in this model, FcεRI and FcγRIII both contribute to AD but differentially regulate immune responses associated with the disease (Abboud, Staumont-Salle et al. 2009). Ando and colleagues developed a mouse model of AD in which eczematous skin lesions are induced by repeated epicutaneous applications of house dust mite extract and staphylococcal enterotoxin B (Kawakami, Yumoto et al. 2007, Ando, Matsumoto et al. 2013). The global skin gene expression pattern in this model was very similar to that observed in human AD skin. Mast cell-deficient mice had markedly reduced skin inflammation; and FcεRI expression was required to attain maximal clinical scores in this AD model (Ando, Matsumoto et al. 2013). However, some features of the model were reduced in mast cell-deficient mice but not in *FcεRI*^{-/-} mice, which suggests that mast cells can amplify inflammation in the context of AD model though both IgE-dependent and IgE-independent pathways (Ando, Matsumoto et al. 2013).

4.1.5. Chronic spontaneous urticaria

Chronic spontaneous urticaria (CSU; also known as chronic idiopathic urticaria) is defined as itchy wheals, angioedema, or both that reoccur for more than 6 weeks without a specific trigger (Zuberbier, Aberer et al. 2014). Antihistamines show clinical benefit for many (but not all) CSU patients, and it is therefore believed that skin mast cells, which are a major source of histamine, play an important role in CSU (Vonakis and Saini 2008). CSU

patients often have high levels of total IgE (Kessel, Helou et al. 2010). However, CSU may not be triggered by specific external antigens. By contrast, most CSU patients exhibit autoimmune responses in the form of serum IgE to autoantigens or IgG autoantibodies to IgE or FcεRI (reviewed in (Kolkhir, Church et al. 2017)). 35-45% of adults with CSU develop a wheal when injected intradermally with their own serum, a test called autologous serum skin test (ASST) (Metz, Gimenez-Arnau et al. 2009). Such positive ASSTs responses have been linked to IgG autoantibodies directed against the high-affinity IgE receptor FcεRI, or less commonly against IgE (Hide, Francis et al. 1993, Chang, Chen et al. 2015, Auyeung, Mittag et al. 2016). Both types of autoantibodies can trigger activation of mast cells (and other FcεRI-bearing cells) through cross-linking of FcεRI. In a recent study, autoreactive T cells specific for FcεRI were also detected in the blood of a large proportion of patients with CSU (Auyeung, Mittag et al. 2016). The authors therefore proposed that, as for other autoimmune diseases, activation of autoreactive T cells is likely one of the initial events in CSU (Auyeung, Mittag et al. 2016). Moreover, some CSU patients have high titers of autoreactive IgE directed against dsDNA or thyroid antigens, such as thyroperoxidase (TPO) (Altrichter, Peter et al. 2011, Hatada, Kashiwakura et al. 2013). It was also recently reported that IL-24 is a common autoantigen in patients with CSU (Schmetzer, Lakin et al. 2017). Such IgE autoantibodies could mediate skin reactions in CSU by inducing mast cell degranulation in response to autoantigens (Altrichter, Peter et al. 2011, Hatada, Kashiwakura et al. 2013, Chang, Chen et al. 2015). It should be noted, however, that the presence of IgE against autoantigens is also documented in diseases other than CSU, such as atopic dermatitis (reviewed in (Hradetzky, Werfel et al. 2015)), and a direct link between autoantibodies and the clinical manifestations of CSU has not yet been demonstrated. Some reports also indicate the presence of IgE against exogenous antigens, such as *Staphylococcus aureus* enterotoxins, in some CSU patients, which could contribute to the pathogenesis of CSU in a subpopulation

of patients (Ye, Hur et al. 2008, Altrichter, Hawro et al. 2018).

In support of a key role of IgE and FcεRI in CSU, the anti-IgE therapeutic antibody omalizumab is now approved for the treatment of CSU (Maurer, Rosen et al. 2013, Chang, Chen et al. 2015, Zhao, Ji et al. 2016). Moreover, most patients with CSU who stop omalizumab treatment relapse within a few months, and a recent study indicates that total IgE serum levels before omalizumab treatment correlate negatively with the time to relapse in these patients (Ertas, Ozyurt et al. 2017). As reviewed in detail by Chang and colleagues (Chang, Chen et al. 2015), the clinical benefits of omalizumab are likely due to a direct blockade of IgE antibodies before they can bind FcεRI and activate mast cells (especially in patients with autoreactive IgE), and/or a downregulation of FcεRI on the surface of mast cells and other effector cells (Chang, Chen et al. 2015).

4.2. Protective roles of IgE

IgE and the main FcεRI-expressing effector cells, mast cells and basophils, do not only play roles in pathology, but also critically contribute to host defense. This has been convincingly demonstrated using mouse models of host defense against certain parasites and venoms.

4.2.1. Host defense against parasites

Helminth infections are generally associated with a "type 2" immune response, characterized by helper type 2 T (T_H2) cells that typically produce IL-4, IL-5 and IL-13, increased numbers of tissue mast cells and eosinophils, and elevated levels of antigen-specific

and unspecific IgE (Finkelman, Shea-Donohue et al. 1997, Anthony, Rutitzky et al. 2007, Grencis, Humphreys et al. 2014). Data from epidemiological studies in humans point towards a protective role for IgE in helminth infections, as increased levels of helminth-specific IgE correlate with host resistance (Hagan, Blumenthal et al. 1991, Rihet, Demeure et al. 1991, Faulkner, Turner et al. 2002). Remarkably, anti-IgE antibody treatment of human patients at high risk of helminth infections did modestly increase parasite infection risk, albeit an effect that did not reach statistical significance (Cruz, Lima et al. 2007). Increased IgE levels might, however, simply reflect a strong T_H2 cell response in infected individuals, the latter being of unquestionable importance in host defense against parasites. Indeed, the actual contributions of non-specific vs. specific IgE antibodies in host defense and parasite clearance are still unclear and numerous experimental studies aiming at addressing this question have led to different, sometimes opposing, conclusions (recently reviewed in (Mukai, Tsai et al. 2016)). Also, protective vs. detrimental roles of IgE antibodies in anti-parasite immunity appear to be parasite-dependent. For instance, data from experiments with IgE-deficient mice indicate beneficial functions for IgE in models of *Trichinella spiralis* (Gurish, Bryce et al. 2004), *Schistosoma mansoni* (King et al. 1997), *Brugia Malayi* (Spencer et al. 2003), *Nippostrongylus brasiliensis* and *Heligmosomoides polygyrus* (Schwartz, Turqueti-Neves et al. 2014). On the other hand, experiments with IgE- or FcεRIα-deficient mice in other studies showed no effect or *decreased* parasite burden in infections with *H. polygyrus* (McCoy, Stoel et al. 2008), *Strongyloides venezuelensis* (Matsumoto, Sasaki et al. 2013) or *S. mansoni* (Jankovic, Kullberg et al. 1997). Among the factors potentially contributing to these discrepancies, one could cite differences in experimental approaches (transgenic [IgE- or FcεRIα-deficient mice] or pharmacological [anti-IgE treatments]), the experimental model and/or the genetic background of the mice (Mukai, Tsai et al. 2016).

4.2.2. Host defense against venoms

Toxic substances, such as venoms, represent an obvious threat for mammals, against which defense mechanisms are needed. In 1991, Margie Profet proposed a theory known as the "toxin hypothesis", suggesting that allergic immune responses (*i.e.*, IgE-associated type 2 immune responses and effector cell-mediated allergic reactions) represent an immunological defense against toxins (Profet 1991). According to this theory, the purpose of an acute allergic reaction (manifested by, *e.g.*, scratching, vomiting, diarrhea, and, in extreme cases, anaphylaxis) is to respond rapidly and avoid, eliminate and/or neutralize toxic substances indicative of life-threatening situations (Profet 1991, Palm, Rosenstein *et al.* 2012).

Recently, Profet's hypothesis was supported by experimental evidence demonstrating that IgE antibodies could contribute to acquired resistance against honeybee and snake venoms (Marichal, Starkl *et al.* 2013, Palm, Rosenstein *et al.* 2013, Starkl, Marichal *et al.* 2016). Marichal, Starkl *et al.* characterized the immune response of mice following subcutaneous injection of whole bee venom to mimic bee stings (Marichal, Starkl *et al.* 2013). The venom induced a robust adaptive type 2 immune response associated with development of venom-specific T_H2 cells and IgE, and this acquired immune response was associated with increased resistance of mice (quantified by survival and body temperature) against a subsequent challenge with bee venom. Experiments involving passive immunization and transgenic animals deficient in IgE or FcεRI demonstrated that IgE antibodies and IgE effector mechanisms played a crucial role in mediating acquired host resistance against bee venom (Marichal, Starkl *et al.* 2013). In a complementary study, Palm, Rosenstein *et al.* provided experimental evidence that a type 2 immune response directed against the bee venom component phospholipase A2 (PLA2) was able to confer protection against a

subsequent near lethal dose of PLA₂, and that such protection was dependent on FcεRI (Palm, Rosenstein et al. 2013). Subsequently, Starkl, Marichal *et al.* found that IgE effector mechanisms also played a critical role in acquired host defense against the venom of the Russell's viper (Starkl, Marichal et al. 2016).

The strong evidence for the important protective function of IgE and IgE effector cells in immune defense against venoms in mice challenges the current view of the function of IgE in (venom-) allergic humans (Artis, Maizels et al. 2012). Therefore, future investigations are needed to determine whether IgE-associated responses can enhance resistance to other toxins, and to understand why, in some species or individuals, exposure to the same venom or venom component may induce either a protective IgE-dependent adaptive immune response, as in the mouse studies described above (Marichal, Starkl et al. 2013, Palm, Rosenstein et al. 2013, Starkl, Marichal et al. 2016), or a deleterious and potentially fatal allergic reaction (*i.e.*, anaphylaxis) (Saelinger and Higginbotham 1974, Charavejasarn, Reisman et al. 1975). This question is of great interest and relevance for basic and clinical allergy research.

5. Targeted anti-IgE therapies

5.1 Anti-IgE antibodies

5.1.1. Omalizumab

Omalizumab is a recombinant humanized IgG1 monoclonal antibody directed against human IgE sold by Novartis and Genentech under the trade name Xolair® (Presta, Lahr et al. 1993). It binds to the Cε3 domain of free IgE, and thereby impairs binding of IgE to both

FcεRI and CD23 (Chang, Davis et al. 1990, Selb, Eckl-Dorna et al. 2016, Davies, Allan et al. 2017) (**Figure 5**). Importantly, omalizumab does not recognize IgE already bound to FcεRI or CD23, and therefore cannot induce cell activation by crosslinking of IgE receptors (Chang, Davis et al. 1990, Davies, Allan et al. 2017).

The IgE binding site of omalizumab has been characterized recently by molecular modeling and crystallography (Zheng, Li et al. 2008, Wright, Chu et al. 2015, Pennington, Tarchevskaya et al. 2016, Davies, Allan et al. 2017). Omalizumab binds to symmetric sites on the two IgE Cε3 domains: it does not directly mask the FcεRI binding site on IgE, but rather induces major conformational changes in the Cε3 domains that inhibit interaction with FcεRI (Zheng, Li et al. 2008, Wright, Chu et al. 2015, Pennington, Tarchevskaya et al. 2016, Davies, Allan et al. 2017). Davies and colleagues reported that, furthermore, IgE binding to CD23 is sterically hindered by Omalizumab due to overlapping binding sites on each Cε3 domain (Davies, Allan et al. 2017). While omalizumab is alleged to be unable to bind IgE already bound to FcεRI, *in vitro* data suggest that omalizumab could also facilitate the dissociation of FcεRI-bound IgE (Eggel, Baravalle et al. 2014).

The first randomized, double blind, placebo controlled trials were conducted in 1996 to assess the tolerability and efficiency of omalizumab in patients with allergic asthma (Boulet, Chapman et al. 1997, Fahy, Fleming et al. 1997). These trials showed a reduction of free serum IgE levels (but an increase in total serum IgE, *i.e.* free IgE and IgE complexed with omalizumab), and improved responses to inhaled allergens following omalizumab therapy ((Boulet, Chapman et al. 1997, Fahy, Fleming et al. 1997). In addition to the reduction of free serum IgE levels, treatment with omalizumab also induced a decrease in the expression of FcεRI on the surface of basophils, DCs and mast cells (Saini, MacGlashan et al.

1999, Prussin, Griffith et al. 2003, Lin, Boesel et al. 2004). In 2003, Xolair® was approved for the treatment of moderate to severe persistent allergic asthma, and is now also approved for the treatment of chronic spontaneous urticaria (CSU) (Maurer, Rosen et al. 2013, Chang, Chen et al. 2015, Zhao, Ji et al. 2016). In addition, more than 150 clinical trials of omalizumab are now listed on the website clinicaltrials.gov, in various diseases including food and venom allergies (in combination with allergen-specific immunotherapy), allergic rhinitis or mastocytosis. It is, however, important to note that, although Xolair® is generally well tolerated, it can induce side effects ranging from skin inflammation (at the site of subcutaneous injection) to systemic anaphylaxis (in 0.1-0.2% of patients) (Harrison, MacRae et al. 2015, Lieberman, Umetsu et al. 2016).

5.1.2. *Ligelizumab*

Ligelizumab (QGE031) is a more recent humanized anti-IgE antibody developed by Novartis. It is also directed against Cε3, but is designed to achieve improved IgE suppression, with an equilibrium dissociation constant (K_D) of 139 pM (as compared to the K_D of omalizumab, ~6-8nM) (Arm, Bottoli et al. 2014) (**Figure 5**). The first clinical results of ligelizumab treatment indicated that this antibody can reduce free-IgE and basophil FcεRI with an efficiency superior to that of omalizumab (NCT01716754). Although the authors did not observe serious adverse events in this study, one patient treated with ligelizumab developed systemic symptoms (Arm, Bottoli et al. 2014). In 2016, ligelizumab was tested in patients with mild allergic asthma, and was shown to have greater efficacy than omalizumab on inhaled and skin allergen responses in these patients (NCT01703312) (Gauvreau, Arm et al. 2016). However, in a more recent phase II field study of asthma patients, ligelizumab was

not seen to be superior to omalizumab (NCT01716754), and further development for asthma has been discontinued.

5.1.3. Quilizumab

Quilizumab (MEMP1972A) is a humanized monoclonal antibody developed by Genentech targeting the M1' epitope which is present on membrane IgE (mIgE) but not on serum IgE (**Figure 5**). Brightbill and colleagues demonstrated, using genetically modified mice that contained the human M1' domain inserted into the mouse IgE locus, that quilizumab could reduce serum IgE and deplete IgE-producing plasma cells *in vivo*, without affecting other immunoglobulin isotypes (Brightbill, Jeet et al. 2010). Quilizumab has been tested in clinical trials in patients with allergic rhinitis (NCT01160861) and mild allergic asthma (NCT01196039) (Gauvreau, Harris et al. 2014). In both studies, reductions in total and allergen-specific serum IgE were observed, as well as improved clinical responses to allergen, suggesting that targeting mIgE can reduce IgE production in humans (Gauvreau, Harris et al. 2014). In a subsequent trial (NCT01582503), treatment with quilizumab also reduced total and allergen-specific IgE in patients with allergic asthma uncontrolled by standard therapy. However, treatment with quilizumab had no impact on asthma exacerbations, lung functions, or patient-reported symptoms in this trial (Harris, Maciucă et al. 2016). Similarly, quilizumab reduced IgE levels by about 30% in CSU patients, but it did not lead to clinical improvements in patient's self-reported itch-severity scores (NCT01987947) (Harris, Cabanski et al. 2016).

5.1.4. XmAb7195

XmAb7195 is a monoclonal anti-IgE antibody developed by Xencor through humanization, affinity maturation, and Fc engineering of the murine parental antibody of omalizumab (MaE11) (Chu, Horton et al. 2012). XmAb7195 has an IgE-binding affinity 5.3-fold higher than that of omalizumab. In addition, two point mutations in the IgG1 Fc portion of the mAb (G236R and L328R) increase the binding affinity to inhibitory IgG receptor FcγRIIB by 400 times compared to omalizumab (Chu, Horton et al. 2012). The authors demonstrated that XmAb7195 could block free IgE and inhibit IgE production in B cells through co-engagement of mIgE and FcγRIIB (Chu, Horton et al. 2012) (**Figure 5**). In a first-in-human phase 1a trial in healthy volunteers (NCT02148744), XmAb7195 decreased IgE levels below the limit of detection in 90% of subjects that had detectable IgE levels at baseline. Transient thrombocytopenia was observed at a dose of 3.0 mg/kg, but no other major adverse events were reported (Gershman, Goldwater et al. 2016). A phase 1b study on the safety, tolerability and bioavailability of a subcutaneous formulation of XmAb7195 has been recently completed (NCT02881853), but the results of this study have not yet been reported.

5.1.5. *MEDI4212*

MEDI4212 is a human IgG1 anti-IgE antibody developed by MedImmune. MEDI4212 was generated using phage display technology, combined with targeted mutagenesis of V_H and V_L sequences to increase its affinity for IgE (Cohen, Dobson et al. 2014). Like omalizumab, MEDI4212 does not recognize IgE already bound to FcεRI, but the authors report that MEDI4212 binds free IgE with an affinity of 1.95 pM, more than 100-fold higher than omalizumab (Cohen, Dobson et al. 2014) (**Figure 5**). Analysis of the crystal structure of IgE Cε3-4 domains in complex with MEDI4212 Fab portion revealed that MEDI4212

recognizes residues in the C ϵ 3 and C ϵ 4 domains, and targets critical residues in C ϵ 3 also involved in binding to Fc ϵ RI. This suggests that MEDI4212 directly competes with Fc ϵ RI for IgE binding (Cohen, Dobson et al. 2014).

Since MEDI4212 recognizes residues in the IgE C ϵ 3-4 domains, it can also bind mIgE on the surface of B cells. MEDI4212 was further engineered in order to increase its potential to eliminate IgE-expressing B cells through antibody-dependent cell-mediated cytotoxicity (ADCC) (Nyborg, Zacco et al. 2016). The authors chose to insert mutations in the Fc portion of MEDI4212 in order to improve its affinity for the IgG receptor Fc γ RIIIA, as ADCC can be performed by natural killer (NK) cells that express Fc γ RIIIA. Indeed, *in vitro* experiments revealed that, thus Fc-engineered, MEDI4212 could eliminate class-switched human IgE B cells more efficiently (Nyborg, Zacco et al. 2016). A phase I study on the pharmacokinetics, pharmacodynamics, and safety of MEDI4212 in subjects with atopy was initiated in 2012 (NCT01544348); and demonstrated that MEDI4212 rapidly reduced free IgE to a greater extent than omalizumab. However, recovery of free IgE to baseline was much faster in patients receiving MEDI4212 as compared as omalizumab, which was attributed to a rapid decrease of serum MEDI4212. Since then, no other study has been initiated with this antibody.

5.2. Anti-IgE, anti-Fc ϵ RI and anti-CD23 DARPins

Designed ankyrin repeat proteins (DARPins) are engineered small proteins that can recognize targets with high specificity and with affinity in the low nanomolar range (Binz, Amstutz et al. 2004, Pluckthun 2015). In 2009, Eggel and collaborators reported identification of two monovalent DARPins, termed B-A4-85 and C-A3-30, displaying high affinity for two

different epitopes on human FcεRIα (Eggel, Baumann et al. 2009). They further produced a bispecific anti-FcεRIα DARPIn (designated 30/85) by linking sequences of the two monovalent DARPins with a [Gly₄–Ser]₄ linker. Remarkably, this bispecific DARPIn showed greater affinity than IgE for FcεRIα, and was able to inhibit IgE–FcεRIα interaction and IgE-mediated degranulation of rat basophilic leukemia cells expressing human FcεRIα (RBL-2H3-huα cells), with an effect similar to that of omalizumab (Eggel, Baumann et al. 2009) (**Figure 5**).

Using a similar strategy, the same group reported identification of several DARPins binding human IgE (**Figure 5**). Among these, the DARPins E2_79 and E3_54 were able to inhibit binding of IgE to either FcεRIα or omalizumab, and inhibit IgE-mediated activation of RBL-2H3-huα cells with higher efficacy than omalizumab (Baumann, Eggel et al. 2010). It was further demonstrated that E2_79 not only prevented binding of free IgE to FcεRI, but also actively disrupted pre-formed IgE:FcεRI complexes (Kim, Eggel et al. 2012). Such facilitated IgE dissociation was observed both *in vitro*, *ex vivo* in primary human basophils, and *in vivo* in human FcεRI transgenic mice (Kim, Eggel et al. 2012, Eggel, Baravalle et al. 2014), suggesting that anti-IgE DARPins might be suitable drug candidates to desensitize allergic patients.

Another DARPIn (E3_53) can recognize both free IgE and IgE bound to FcεRI. This DARPIn was linked to the Fc portion of human IgG1 (using a [Gly₄–Ser]₃ linker) to produce a fusion protein capable of cross-linking FcεRI-bound IgE with the inhibitory receptor FcγRIIB. This molecule, termed DE53-Fc, was able to reduce allergen-induced basophil activation *ex vivo* using whole blood samples from allergic patients (Eggel, Buschor et al. 2011). Furthermore, by using blocking antibodies against FcγRIIB, the authors demonstrated

that binding of DE53-Fc to Fc γ RIIB was required for full inhibitory properties of the fusion molecule (Eggel, Buschor et al. 2011). Confirming this mode of action, it was later reported that mutant forms of DE53-Fc displaying enhanced affinity for Fc γ RIIB also have greater capacity to inhibit basophil activation (Buschor, Eggel et al. 2014). However, while mouse basophils and mast cells and human basophils express high levels of Fc γ RIIB, it is still ambiguous whether human mast cells also express this inhibitory receptor (Zhao, Kepley et al. 2006). Therefore, whether cross-linking of Fc ϵ RI-bound IgE to Fc γ RIIB could inhibit IgE- and mast cell-mediated responses in humans remains an open question.

More recently, two DARPins (D86 and D89), which specifically recognize CD23, were also identified. These anti-CD23 DARPins inhibited binding of IgE to CD23 (which suggests that they share a similar binding epitope to IgE), and could inhibit IgE synthesis in human peripheral B cells (Fellmann, Buschor et al. 2015).

5.3. Fc ϵ -Fc γ fusion proteins

The human Fc γ -Fc ϵ bifunctional fusion protein consists of the Fc region of human IgG1 (hinge-C γ 2-3) linked to the Fc portion of human IgE (C ϵ 2-4) by a 15 amino acid linker (Gly₄Ser)₃ (Zhu, Kepley et al. 2002). As first described by Zhu *et al.*, this fusion protein (called GE2) was able to compete with IgE for the binding to Fc ϵ RI, and could thereby be used to ‘desensitize’ mast cells and basophils (**Figure 5**). It could also bind to IgG Fc γ Rs through its C γ 2-3 domains, and it was therefore proposed that GE2 could block IgE-mediated mast cell and basophil activation through co-engagement of Fc ϵ RI with the inhibitory receptor Fc γ RIIB (Zhu, Kepley et al. 2002). Indeed, the authors demonstrated that GE2 was able to inhibit histamine release in primary human blood basophils sensitized with IgE, and

could also block IgE-mediated passive cutaneous anaphylaxis (PCA) in transgenic mice expressing human FcεRI (Zhu, Kepley et al. 2002). In addition to its effect on mast cells and basophils, it was proposed that the fusion protein could also inhibit allergic inflammation through effects on FcεRI-expressing Langerhans cells (Kepley, Zhang et al. 2003), and inhibit IgE class switch recombination in B cells by co-aggregating CD23 and FcγRII (Yamada, Zhu et al. 2003). Several attempts were subsequently made to improve the efficiency of the fusion protein, such as removal of the (Gly₄Ser)₃ linker, or mutations in the Cγ portion to improve binding to FcγRIIB and/or decrease binding to FcγRIII (Allen, Kepley et al. 2007). However, most of these modifications altered the effectiveness of the fusion protein to inhibit FcεRI-mediated functions (Allen, Kepley et al. 2007). Nevertheless, and as described above (part 5.2), while basophils undoubtedly express FcγRIIB, it is still unclear whether human mast cells express FcγRIIB *in vivo* (Zhao, Kepley et al. 2006).

The effects of GE2 were also tested in non-human primates. Rhesus monkeys have been reported to exhibit skin test reactivity and serum IgE directed against dust mites (Schelegle, Gershwin et al. 2001, Zhang, Kepley et al. 2004). Taking advantage of this, Zhang and collaborators showed that GE2 was able to inhibit dust mite allergen-induced skin reactivity in rhesus monkeys in a dose-dependent manner (Zhang, Kepley et al. 2004). In a subsequent study, GE2 demonstrated efficacy in a model of house dust mite-induced allergic asthma in cynomolgus monkeys (Van Scott, Mertsching et al. 2008). The effects of GE2 lasted for 4 weeks and were associated with reduced numbers of circulating basophils and reduced FcεRI expression on basophils. However, repeated injections of GE2 induced the production of serum antibodies against the fusion protein, and increased occurrence of serious adverse events, including anaphylaxis (Van Scott, Mertsching et al. 2008).

6. Concluding remarks

Discovered some 50 years ago, IgE continues to be the focus of extensive academic and industrial research. The clinical benefits of the anti-IgE antibody omalizumab best exemplify the key role of IgE in allergic diseases and chronic spontaneous urticaria. Besides omalizumab, several new anti-IgE therapies are now at various stages of clinical development, with some promising early results. Recent insights from crystallographic studies have also shed light on the mechanisms by which IgE antibodies recognize their main receptors FcεRI and CD23; findings that should help in the design of additional therapeutic approaches aimed at blocking these interactions.

While IgE can undeniably trigger allergic reactions, it is also now clear that not all allergies are IgE-mediated, and evidence from mouse models suggests that IgE may have protective functions in host defense against parasites and venoms. An ongoing effort is therefore necessary to clearly identify the full spectrum of IgE-mediated diseases, but also to address the potential limitations of targeted anti-IgE therapies.

7. Acknowledgments

The authors thank Caitlin Gillis (VIB-UGent Center for Inflammation Research, Belgium) for critical reading of our manuscript. B.B. is supported by a stipend from the Pasteur - Paris University (PPU) International Ph.D. Program. E.C. is supported by a CIFRE fellowship from ANRT (Association Nationale de la Recherche et de la Technologie). T.M. is supported by a FRFS-WELBIO Starting Grant and by the Acteria Foundation, and is a research associate of the F.R.S.-FNRS. P.S. is supported by a Marie Skłodowska-Curie Individual Fellowship of

the European Commission (H2020-MSCA-IF-2014 655153). L.L.R. acknowledges support from the European Commission Marie Skłodowska-Curie Individual Fellowship (H2020-MSCA-IF-2014 656086) and the French ‘Institut National de la Santé et de la Recherche Médicale’ (INSERM).

8. Conflict of Interest Statement

E.C. is an employee of Neovacs SA. L.L.R. reports serving as consultant for Neovacs SA. All other authors have declared no conflicts of interest.

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Figure legends

Figure 1. IgE structure. IgE antibodies consist of two identical heavy chains (composed of a variable V_H domain and four constant $C\epsilon$ domains) and two identical light chains (composed of a variable V_L domain and a constant C_L domain). ‘Fab’: region responsible for antigen recognition and binding. ‘Fc’: portion responsible for IgE effector functions. The positions of interdomain disulfide bridges, N-linked glycosylation sites (in human IgE), Fc ϵ RI- and CD23-binding sites are indicated.

Figure 2. Structure of Fc ϵ RI and its interaction with IgE. **a.** Fc ϵ RI is expressed on mast cells and basophils as a tetramer formed with one α subunit, one β subunit and a dimer of disulfide-linked γ subunits. IgE binds the receptor via surface loops in $C\epsilon 3$, with contributions from the $C\epsilon 2$ - $C\epsilon 3$ linker region. **b.** The two $C\epsilon 3$ domains of IgE bind distinct sites on Fc ϵ RI α , one site found in the D2 domain (site 1), and a second site formed by a cluster of four surface-exposed tryptophan residues in the D1-D2 interface (site 2) (Protein Data Bank ID: 2Y7Q).

Figure 3. Conformational changes in IgE Fc portion upon binding to Fc ϵ RI or CD23. The $C\epsilon 3$ domains of free IgE are found in a ‘closed’ conformation in which the Fc ϵ RI α binding site in $C\epsilon 3$ is masked (middle; Protein Data Bank [PDB] ID: 2WQR). $C\epsilon 3$ adopts an ‘opened’ conformation upon binding to Fc ϵ RI, which is incompatible with CD23 binding (left; PDB ID: 1F6A-2). By contrast, $C\epsilon 3$ adopts a ‘closed’ conformation upon binding to CD23, which is incompatible with Fc ϵ RI binding (right; PDB ID: 4GKO).

Figure 4. Structure of CD23 and its interaction with IgE. **a.** CD23 self-associates as a trimer, and is composed of an IgE-binding ‘head domain’ (which belongs to the C-type lectin superfamily) linked to the membrane by an extracellular coiled-coil stalk region, and a small cytoplasmic N-terminal domain. **b.** The IgE binding site of CD23 is located in the C-terminal head domain (in green), with some additional contributions from the stalk region (not shown). Two CD23 molecules bind to each IgE heavy chain, primarily to the Cε3 domains but with a contribution from Cε4 (Protein Data Bank ID: 4GKO).

Figure 5. Key role of IgE in allergic reactions. Stimulation with the T_H2 cytokines IL-4 and IL-13 induces class-switching of B cells into IgE-producing cells. IgE binds to its high-affinity receptor FcεRI on the surface of tissue mast cells and blood basophils. Upon exposure to an allergen, in allergic patients, allergen recognition by allergen-specific IgE on the surface of mast cells and basophils induces crosslinking of FcεRI, leading to degranulation and the immediate release of histamine, proteases and other preformed mediators, as well as *de novo* synthesis of lipid mediators (prostaglandins, leukotrienes,...), cytokines and chemokines. These mediators can act locally or systemically, leading to the clinical features of immediate hypersensitivity, such as bronchoconstriction, urticaria, diarrhea (when acting locally in the airways, the skin and the gut, respectively) and vasodilatation. These mediators are also responsible for late-phase allergic responses, entailing the recruitment of leukocytes, mainly eosinophils and neutrophils. Several drugs have been developed to counteract the effects of IgE. These drugs either target IgE production, block free IgE or compete with IgE for binding to FcεRI. The only FDA-approved anti-IgE drug is Omalizumab, a humanized anti-IgE mAb that blocks free IgE, and which is approved for the treatment of moderate to severe persistent allergic asthma, and chronic spontaneous urticaria (CSU). Ag: antigen.

Figure 1

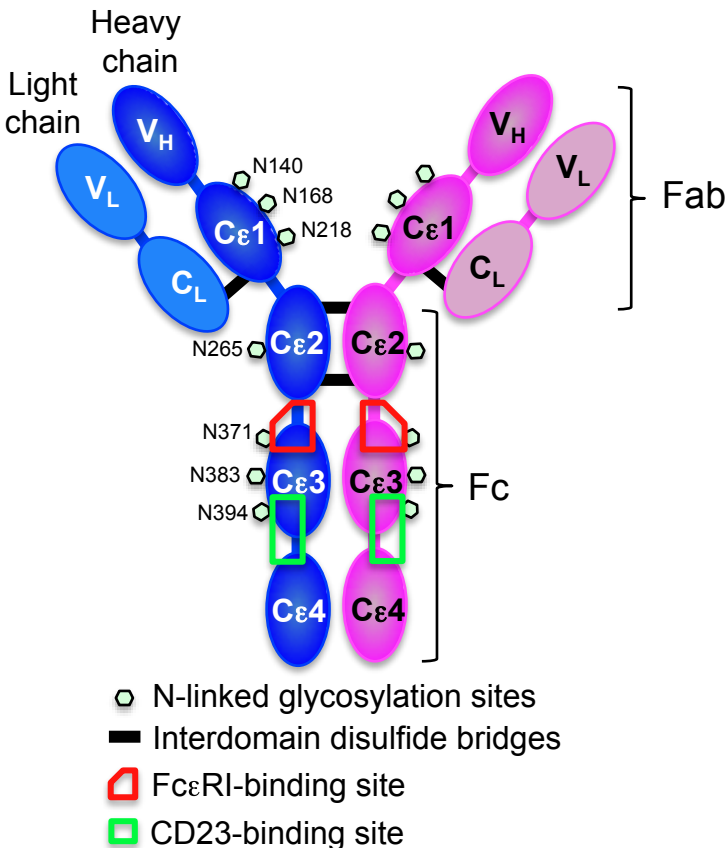
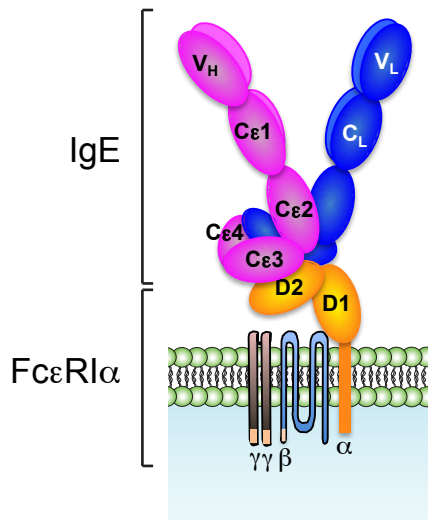


Figure 2

a



b

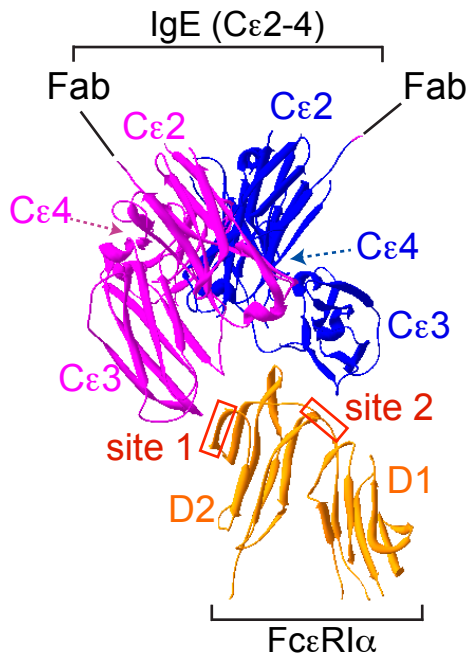
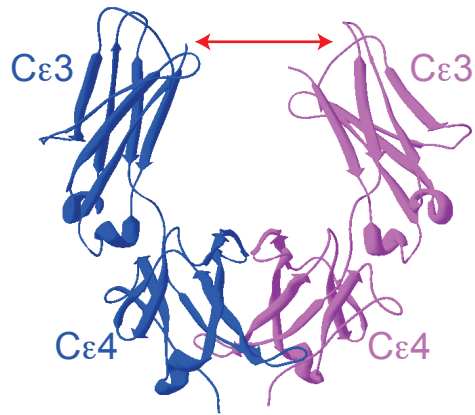


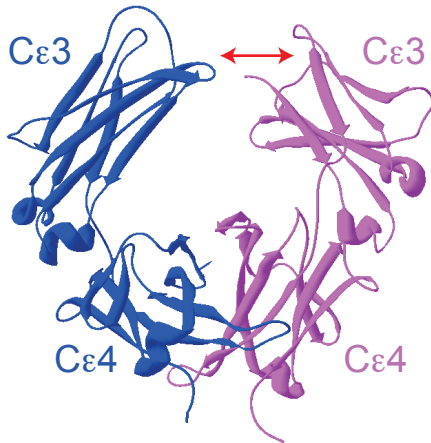
Figure 3

'Open' conformation

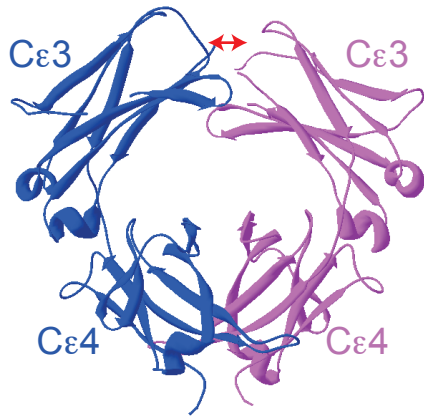


Fc ϵ R1-bound IgE

'Closed' conformations



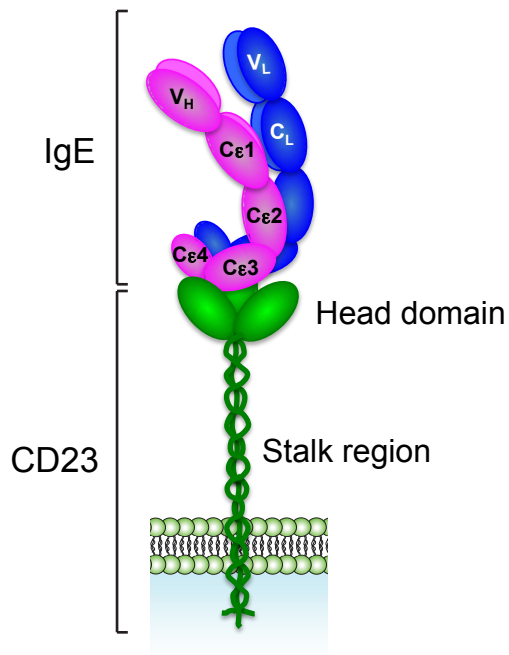
Free IgE



CD23-bound IgE

Figure 4

a



b

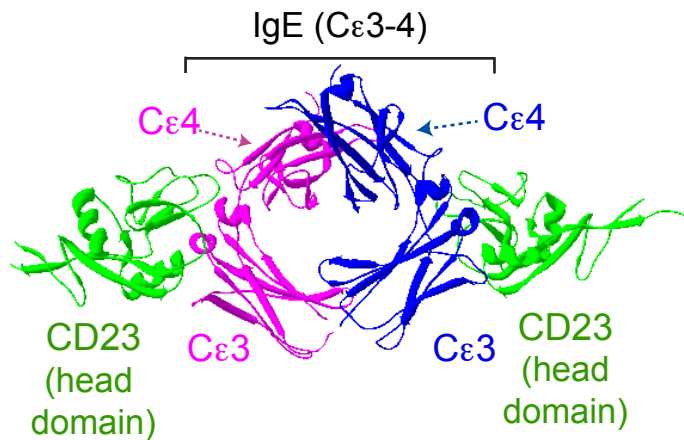


Figure 5

