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; **Cε**: constant epsilon domain of IgE; **C_L**: constant region of an antibody's light chains; **CSU**: chronic spontaneous urticaria; **DARPins**: 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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96 # 1. Introduction

97

98 Immunoglobulin E (IgE) was discovered about 50 years ago. In 1966, the Ishizakas'
99 group in Japan described an immunoglobulin different from the known immunoglobulin
100 classes, that could induce allergic reactions in the skin, and which they called γE antibody
101 (Ishizaka and Ishizaka 1967). During the same period, the group of Johansson and Bennich in
102 Sweden isolated a new immunoglobulin class, which they called IgND (Johansson and
103 Bennich 1967). It soon turned out that γE and IgND belong to the same and unique antibody
104 class, and the official name IgE was given in 1968 (Bennich, Ishizaka et al. 1968). The story
105 behind this discovery has been the subject of many reviews, including two recent reviews by
106 the discoverers themselves (Ishizaka and Ishizaka 2016, Johansson 2016). IgE is the isotype
107 found at the lowest concentration in the circulation (50-200 ng/ml IgE in healthy individuals
108 *vs*. ~10 mg/ml for IgG) (Dullaers, De Bruyne et al. 2012). However, IgE levels can increase
109 dramatically in individuals with allergic diseases (Galli and Tsai 2012, Platts-Mills, Schuyler
110 et al. 2016). Indeed, the importance of IgE in allergy was demonstrated at the time of its
111 discovery, when the investigators identified that purified IgE was capable of transferring skin
112 reactivity from sensitized human subjects to naive hosts (Ishizaka and Ishizaka 2016,
113 Johansson 2016). This discovery has had great importance for both the diagnosis and
114 treatment of allergic disorders: quantification of allergen-specific IgE is one of the main
115 diagnostic criteria for allergies (Hamilton, MacGlashan et al. 2010), and the anti-IgE
116 therapeutic antibody omalizumab is now approved for the treatment of moderate to severe
117 persistent allergic asthma, and shows great potential for the treatment of other allergic
118 diseases (Humbert, Busse et al. 2014, Pelaia, Vatrella et al. 2015, Kawakami and Blank
119 2016). Omalizumab has also been approved for the treatment of chronic spontaneous urticaria

120 (CSU), demonstrating that the pathologic functions of IgE extend beyond allergy (Maurer,
121 Rosen et al. 2013, Chang, Chen et al. 2015, Zhao, Ji et al. 2016).
122
123 IgE antibodies exist in two forms: a membrane-bound form (mIgE) expressed by B cells
124 that have undergone class switching to IgE, and a secreted form produced by plasma B cells.
125 mIgE serves as a B cell receptor involved in antigen uptake and presentation. The structure
126 and functions of mIgE, as well as the regulation of IgE synthesis, have been extensively
127 reviewed elsewhere (Geha, Jabara et al. 2003, Gould and Sutton 2008, Wu and Zarrin 2014).
128 This review will focus mainly on the effector functions of secreted IgE (hereafter referred to
129 as 'IgE').
130
131 IgE exerts its biological functions by binding to two main receptors: FcεRI and CD23
132 (FcεRII). The high affinity IgE receptor, FcεRI, is expressed on the surface of blood basophils
133 and tissue resident mast cells; and on other cell types in humans (but not in mice), including
134 neutrophils, eosinophils, platelets, monocytes and dendritic cells (Kraft and Kinet 2007). The
135 low affinity receptor CD23 is expressed mainly by B cells (Sutton and Davies 2015), but also
136 by several other cell populations including neutrophils, eosinophils, follicular DCs and
137 intestinal epithelial cells (IECs) (Acharya, Borland et al. 2010). CD23 on B cells serves
138 mainly as a negative regulator of IgE synthesis (Acharya, Borland et al. 2010). Crosslinking
139 of FcεRI-bound IgE can initiate allergic reactions by inducing the activation of mast cells and
140 basophils, the immediate release of preformed granule-stored mediators such as histamine and
141 proteases, and the *de novo* production of lipid mediators (*e.g.* prostaglandins, leukotrienes),
142 cytokines and chemokines (Galli, Kalesnikoff et al. 2005, Voehringer 2013, Wernersson and
143 Pejler 2014).
144

In this review, we will describe our current understanding of the interactions between IgE and its receptors FcεRI and CD23. We will review the known and potential functions of IgE antibodies in health and disease, in particular their detrimental roles in allergic diseases and chronic spontaneous urticaria, as well as their 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.

2. IgE structure

IgE antibodies are composed of two identical heavy chains (each comprising a variable V_H domain and four constant Cε 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 (Cε2). As detailed in part *3.1.3*, this additional Cε2 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 FcεRI (McDonnell, Calvert et al. 2001). The FcεRI binding site is located in the Cε3 domain and in the Cε2-Cε3 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 Cε3 domain, with contributions from the Cε4 domain (described in more detail in part *3.2.3*) (**Figure 1**). The crystal structure of the human Cε3-Cε4 domains revealed that, by rotating relatively to Cε4, Cε3 can adopt either 'open' or 'closed' conformations. This conformational flexibility regulates the binding of IgE to both FcεRI 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 Cε3 domain at asparagine-394 (N394) in humans, and N384 in mouse, abrogates the binding of IgE to FcεRI,

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 ~ 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 (FcεRIα) 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 FcεRIα 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). FcεRIβ has a cytoplasmic immunoreceptor tyrosine-based activation motif (ITAM), which acts as signal amplifier. The FcεRIγ 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 αγ2 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, Lamkhioued et al. 2001), eosinophils (Gounni, Lamkhioued et al. 1994) and platelets (Joseph, Gounni et al. 1997, Hasegawa, Pawankar et al. 1999). It was reported that expression of the αγ2 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 FcεRI (sFcεRI) of about 40 kDa, and which contains an intact IgE binding site, has been described in human serum (Dehlink, Platzer et al. 2011). However, the cell types that release or shed this protein in humans, and the physiological role of sFcεRI, remain to be identified (reviewed in (Platzer, Ruiter et al. 2011).

3.1.2. FcεRI functions

FcεRI plays a key role in mediating the biological functions of IgE *in vivo*, which is best exemplified by the fact that FcεRI-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 αβγ2 FcεRI 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 FcεRIα chain under the control of its own promoter have also given significant insight into the functions of human FcεRI (Dombrowicz, Brini et al. 1996, Dombrowicz, Lin et al. 1998, Greer, Wu et al. 2014). *hFcεRIα*^{Tg} mice (bred on a mouse FcεRI-deficient background) express a 'humanized' FcεRI 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 naive 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 naive 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 ($k_{off} \approx 10^{-5}$ 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

298 contributes to the slow dissociation rate of IgE-FcεRIα complexes through conformational
299 changes rather than direct interactions with the receptor (Holdom, Davies et al. 2011).
300
301 Analysis of the crystal structures of free *vs*. receptor-bound IgE Fc domains have
302 revealed that the Cε3 domains of IgE undergo a large conformational rearrangement upon
303 binding to FcεRI (Wurzburg, Garman et al. 2000, Wan, Beavil et al. 2002, Wurzburg and
304 Jardetzky 2009, Holdom, Davies et al. 2011). The free IgE Fc portion was observed in a
305 'closed' conformation in which the FcεRIα binding site in Cε3 is masked (Wurzburg,
306 Garman et al. 2000, Wan, Beavil et al. 2002, Wurzburg and Jardetzky 2009). This masking is
307 achieved as the Cε2 domains in the free Fc fragment are folded back asymmetrically onto the
308 Cε3 and Cε4 domains, locking the Cε3 domains in a 'closed' conformation (Wan, Beavil et
309 al. 2002) (**Figure 3**). The authors suggest that free 'bent' IgE may first engage FcεRI through
310 only one Cε3 domain, followed by an important conformational change involving Cε2,
311 whereby Cε3 would adopt an 'open' conformation, leading to engagement of the second Cε3.
312
313 **3.2. The low affinity IgE receptor CD23 (FcεRII)**
314
315 *3.2.1. CD23 structure and expression*
316
317 CD23, also known as FcεRII, is the low affinity receptor for IgE (K_d = 10^{-5} M)
318 (Wurzburg, Tarchevskaya et al. 2006). The structure of CD23 and its interaction with IgE
319 have been reviewed in detail (Sutton and Davies 2015). CD23 self-associates as trimer, and is
320 composed of an IgE-binding 'head domain' (which belongs to the C-type lectin superfamily)
321 linked to the membrane by an extracellular coiled-coil stalk region, and a small cytoplasmic
322 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 I* 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, Riffo-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

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

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

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

399 Interestingly, the latter study also demonstrated that mutation of the N-glycosylation site of
400 CD23 (N63) alone is sufficient to enhance binding of IgE (Selb, Eckl-Dorna et al. 2016).
401
402 Vercelli *et al*. first demonstrated, using a bank of peptides spanning the IgE Cε2-4
403 domains, that CD23 recognizes a motif in the Cε3 domain of IgE (Vercelli, Helm et al. 1989).
404 This was confirmed in a study using chimeric IgE molecules in which the human Cε3 domain
405 was replaced by mouse Cε3: these chimeric molecules bound to mouse CD23 and
406 concomitantly lost their ability to bind the human receptor (Nissim, Schwarzbaum et al.
407 1993). Thereafter, the CD23 binding site on IgE was more precisely mapped to the A-B loop
408 of the Cε3 domain (residues 341-356), with a key role for lysine 352 (Sayers, Housden et al.
409 2004). More recently, the crystal structure of the soluble head domain of CD23 bound to a
410 Cε3-4 IgE dimer was resolved by Dhaliwal and collaborators (Dhaliwal, Yuan et al. 2012).
411 These authors found that one CD23 molecule binds to each IgE heavy chain, principally via
412 the Cε3 domains but with a contribution from Cε4 (Dhaliwal, Yuan et al. 2012) (**Figure 4**).
413 Although the binding sites for FcεRI and CD23 are at opposite ends of the Cε3 domain,
414 binding of the two receptors to IgE is mutually exclusive. Indeed, binding of IgE to CD23
415 induces conformational changes in Cε3, leading to a highly 'closed' conformation
416 incompatible with FcεRI binding (Borthakur, Hibbert et al. 2012, Dhaliwal, Yuan et al. 2012).
417 Similarly, the 'opened' conformation adopted by Cε3 upon binding to FcεRI is incompatible
418 with CD23 binding (Borthakur, Hibbert et al. 2012, Dhaliwal, Yuan et al. 2012) (**Figure 3**).
419 Finally, the crystal structure of CD23 bound to a complete IgE Fc fragment was reported,
420 revealing that the IgE Cε2 domain also contributes to CD23 binding, in addition to the
421 known contributions of the Cε3 and Cε4 domains (Dhaliwal, Pang et al. 2017).
422
423 **3.3. Other IgE or FcεRI 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

449 deficient for FcεRI, CD23 and all FcγRs except FcγRIV suggested that the *in vivo*
450 engagement of FcγRIV by IgE immune complexes can synergize with mediators released by
451 IgE-activated mast cells to induce lung inflammation (Mancardi, Iannascoli et al. 2008).
452
453 # 4. Roles of IgE in health and disease
454
455 ## 4.1. Pathologic roles of IgE
456
457 *4.1.1. Immediate hypersensitivity reactions*
458
459 IgE antibodies are probably best known for their critical role in acute allergic
460 reactions. In allergic individuals, mast cells and basophils have antigen-specific IgE bound to
461 FcεRI expressed on the cell surface (Galli and Tsai 2012). Antigen-mediated IgE/FcεRI
462 crosslinking initiates a complex signaling cascade (Reber and Frossard 2014, Sibilano, Frossi
463 et al. 2014), leading to the eventual activation of these effector cells and the immediate and
464 rapid release of preformed granule-stored mediators (Wernersson and Pejler 2014) (*e.g.*,
465 histamine, serotonin, proteoglycans, proteases and cytokines) and *de novo* production and
466 release of an impressive range of lipid mediators (*e.g.*, prostaglandins, leukotrienes),
467 cytokines and chemokines (Galli, Kalesnikoff et al. 2005, Voehringer 2013). These mediators
468 can act locally or systemically, leading to the clinical features of immediate hypersensitivity,
469 such as bronchoconstriction, urticaria, diarrhea (when acting locally in the airways, the skin
470 and the gut, respectively) (**Figure 5**).
471
472 *4.1.2. Anaphylaxis*
473

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

499 marked reduction in the absolute number of circulating basophils, during anaphylactic
500 reactions to *Hymenoptera venom* in humans (Korosec, Turner et al. 2017). While these data
501 suggest that basophils are activated in human anaphylaxis, they do not however demonstrate a
502 significant contribution to anaphylaxis pathophysiology. Even in mice, the role of basophils in
503 IgE-mediated anaphylaxis remains contentious. Different reports indicate that depletion of
504 basophils does not reduce IgE-mediated local or systemic passive anaphylaxis (Wada,
505 Ishiwata et al. 2010, Sawaguchi, Tanaka et al. 2012). Mukai and colleagues reported that
506 intravenous injection of antigen-specific IgE in mice, followed one day later by subcutaneous
507 challenge with the antigen, can induce a triphasic response (Mukai, Matsuoka et al. 2005).
508 The 'immediate' and 'late-phase' (6 to 10 h after challenge) responses were dependent on
509 mast cells. However, the third-phase, beginning one to two days after challenge, was
510 independent of mast cells and was abrogated upon depletion of basophils (Mukai, Matsuoka
511 et al. 2005, Obata, Mukai et al. 2007, Sawaguchi, Tanaka et al. 2012). This third-phase
512 delayed response was also absent in mice lacking FcRγ (a signaling subunit shared by FcεRI
513 and activating IgG Fcγ receptors), and was restored upon engraftment of these mice with
514 basophils purified from WT mice but not from *FcRγ*$^{-/-}$ mice (Mukai, Matsuoka et al. 2005).
515 Since this passive model relies on specific IgE antibodies, and not on IgG, these results
516 strongly suggest that direct activation of basophils through FcεRI is responsible for the
517 delayed allergic skin inflammation observed this model. Using a similar model of IgE-
518 mediated chronic allergic inflammation, Cheng *et al*. also reported markedly reduced
519 eosinophilic dermatitis in basophil-deficient mice as compared to control mice three days
520 after cutaneous challenge with the relevant antigen (Cheng, Sullivan et al. 2015).
521
522 The presence of allergen-specific IgE alone does not explain an individual's
523 susceptibility to allergy and anaphylaxis. Allergen-specific IgE can be detected in subjects

524 who do not develop clinical symptoms when exposed to the corresponding allergen (Sicherer
525 and Sampson 2010). Conversely, some patients can experience near fatal anaphylaxis despite
526 having low or undetectable levels of circulating allergen-specific IgE (Simons, Frew et al.
527 2007), which suggests (but does not prove) the existence of IgE-independent pathways of
528 anaphylaxis in humans (recently reviewed in (Finkelman, Khodoun et al. 2016) and (Reber,
529 Hernandez et al. 2017)). More definitive evidence for IgE-independent pathways of
530 anaphylaxis has been obtained using mouse models of active systemic anaphylaxis (ASA), in
531 which mice are sensitized with an antigen (to produce antigen-specific antibodies) and re-
532 exposed later on to the same antigen to induce anaphylaxis (Finkelman, Khodoun et al. 2016,
533 Munoz-Cano, Picado et al. 2016). Mice deficient for IgE or for FcεRI can still partially (Sun,
534 Arias et al. 2007, Arias, Chu et al. 2011, Balbino, Sibilano et al. 2017) or fully (Oettgen,
535 Martin et al. 1994, Dombrowicz, Flamand et al. 1997, Jonsson, Mancardi et al. 2011) develop
536 features of anaphylaxis in these ASA models. Other studies have subsequently shown that
537 mouse IgG antibodies can trigger anaphylaxis in ASA models, through activation of IgG
538 receptors (FcγRs) on the surface of various myeloid cells, including basophils, macrophages
539 and neutrophils (Miyajima, Dombrowicz et al. 1997, Jonsson, Mancardi et al. 2011, Khodoun,
540 Kucuk et al. 2013, Finkelman, Khodoun et al. 2016, Balbino, Sibilano et al. 2017).
541
542 *4.1.3. Allergic asthma*
543
544 Asthma is a chronic inflammatory disease of the airways with continual increasing
545 prevalence (Busse and Lemanske 2001, Subbarao, Mandhane et al. 2009). In many patients,
546 the asthmatic condition is associated with allergic reactivity to environmental allergens and
547 elevated levels of IgE antibodies (Busse and Lemanske 2001). In these allergic patients, IgE is
548 thought to contribute to the asthmatic manifestations (Galli and Tsai 2012). Following antigen

549 exposure in the airways, rapid local IgE/FcεRI-dependent mast cell activation and the
550 immediate hypersensitivity reaction can lead to increased vascular permeability,
551 bronchoconstriction and increased mucus production. A large array of cytokines, growth
552 factors and chemokines secreted by activated mast cells can influence airway remodeling
553 (Galli, Tsai et al. 2008, Moiseeva and Bradding 2011). Finally, IgE can also act on other cell
554 types that express FcεRI or CD23, such as DCs, B cells, basophils or (in humans) eosinophils,
555 which may potentially affect several biological responses associated with the asthmatic
556 response (Galli, Tsai et al. 2008, Galli and Tsai 2012). Supporting the important role of IgE in
557 asthma, the anti-IgE antibody omalizumab has been shown to reduce asthma symptoms in
558 several clinical trials involving patients with moderate-to-severe and severe allergic asthma
559 (reviewed in (Humbert, Busse et al. 2014)) (for more detail see part *5.1*, below).
560
561 *4.1.4. Atopic dermatitis*
562
563 Eczema, or atopic dermatitis (AD), is a pruritic inflammatory skin disease with
564 dramatically increased incidence over the last decades (Bieber 2008, Dharmage, Lowe et al.
565 2014). AD manifestations are characterized by pruritus (itching), skin inflammatory lesions
566 associated with cellular infiltration and histopathological changes, and atopy. Indeed, the
567 majority of AD patients exhibit increased serum levels of total and antigen-specific IgE
568 (Leung and Bieber 2003, Laske and Niggemann 2004, Oyoshi, He et al. 2009). The function
569 of IgE in development of AD is supported by the beneficial effect of anti-IgE therapy in a
570 number of clinical studies (Belloni, Andres et al. 2008, Liu, Goodarzi et al. 2011).
571
572 Abboud, Staumont-Sallé *et al*. used a mouse model of AD induced by repeated
573 epicutaneous sensitizations with ovalbumin. They reported that several features of this model

574 (including T_H1 and T_H2 skin responses, mast cell recruitment into draining lymph nodes and
575 IgE production) were reduced in *FcεRI*$^{-/-}$ mice. In this model, T_H2 skin response as well as T
576 cell proliferation and IgG1 production were also reduced in mice lacking the IgG receptor
577 FcγRIII (Abboud, Staumont-Salle et al. 2009). In addition, symptoms of AD were completely
578 absent in mice deficient for FcRγ, a subunit shared by FcεRI and FcγRIII (and several other
579 FcR). The authors therefore concluded that in this model, FcεRI and FcγRIII both contribute
580 to AD but differentially regulate immune responses associated with the disease (Abboud,
581 Staumont-Salle et al. 2009). Ando and colleagues developed a mouse model of AD in which
582 eczematous skin lesions are induced by repeated epicutaneous applications of house dust mite
583 extract and staphylococcal enterotoxin B (Kawakami, Yumoto et al. 2007, Ando, Matsumoto
584 et al. 2013). The global skin gene expression pattern in this model was very similar to that
585 observed in human AD skin. Mast cell-deficient mice had markedly reduced skin
586 inflammation; and FcεRI expression was required to attain maximal clinical scores in this AD
587 model (Ando, Matsumoto et al. 2013). However, some features of the model were reduced in
588 mast cell-deficient mice but not in *FcεRI*$^{-/-}$ mice, which suggests that mast cells can amplify
589 inflammation in the context of AD model though both IgE-dependent and IgE-independent
590 pathways (Ando, Matsumoto et al. 2013).
591
592 *4.1.5. Chronic spontaneous urticaria*
593
594 Chronic spontaneous urticaria (CSU; also known as chronic idiopathic urticaria) is
595 defined as itchy wheals, angioedema, or both that reoccur for more than 6 weeks without
596 a specific trigger (Zuberbier, Aberer et al. 2014). Antihistamines show clinical benefit for
597 many (but not all) CSU patients, and it is therefore believed that skin mast cells, which are a
598 major source of histamine, play an important role in CSU (Vonakis and Saini 2008). CSU

599 patients often have high levels of total IgE (Kessel, Helou et al. 2010). However, CSU may
600 not be triggered by specific external antigens. By contrast, most CSU patients exhibit
601 autoimmune responses in the form of serum IgE to autoantigens or IgG autoantibodies to IgE
602 or FcεRI (reviewed in (Kolkhir, Church et al. 2017)). 35-45% of adults with CSU develop a
603 wheal when injected intradermally with their own serum, a test called autologous serum skin
604 test (ASST) (Metz, Gimenez-Arnau et al. 2009). Such positive ASSTs responses have been
605 linked to IgG autoantibodies directed against the high-affinity IgE receptor FcεRI, or less
606 commonly against IgE (Hide, Francis et al. 1993, Chang, Chen et al. 2015, Auyeung, Mittag
607 et al. 2016). Both types of autoantibodies can trigger activation of mast cells (and other
608 FcεRI-bearing cells) through cross-linking of FcεRI. In a recent study, autoreactive T cells
609 specific for FcεRI were also detected in the blood of a large proportion of patients with CSU
610 (Auyeung, Mittag et al. 2016). The authors therefore proposed that, as for other autoimmune
611 diseases, activation of autoreactive T cells is likely one of the initial events in CSU (Auyeung,
612 Mittag et al. 2016). Moreover, some CSU patients have high titers of autoreactive IgE
613 directed against dsDNA or thyroid antigens, such as thyroperoxidase (TPO) (Altrichter, Peter
614 et al. 2011, Hatada, Kashiwakura et al. 2013). It was also recently reported that IL-24 is a
615 common autoantigen in patients with CSU (Schmetzer, Lakin et al. 2017). Such IgE
616 autoantibodies could mediate skin reactions in CSU by inducing mast cell degranulation in
617 response to autoantigens (Altrichter, Peter et al. 2011, Hatada, Kashiwakura et al. 2013,
618 Chang, Chen et al. 2015). It should be noted, however, that the presence of IgE against
619 autoantigens is also documented in diseases other than CSU, such as atopic dermatitis
620 (reviewed in (Hradetzky, Werfel et al. 2015)), and a direct link between autoantibodies and
621 the clinical manifestations of CSU has not yet been demonstrated. Some reports also indicate
622 the presence of IgE against exogenous antigens, such as *Staphylococcus aureus* enterotoxins,
623 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

649 and unspecific IgE (Finkelman, Shea-Donohue et al. 1997, Anthony, Rutitzky et al. 2007,
650 Grencis, Humphreys et al. 2014). Data from epidemiological studies in humans point towards
651 a protective role for IgE in helminth infections, as increased levels of helminth-specific IgE
652 correlate with host resistance (Hagan, Blumenthal et al. 1991, Rihet, Demeure et al. 1991,
653 Faulkner, Turner et al. 2002). Remarkably, anti-IgE antibody treatment of human patients at
654 high risk of helminth infections did modestly increase parasite infection risk, albeit an effect
655 that did not reach statistical significance (Cruz, Lima et al. 2007). Increased IgE levels might,
656 however, simply reflect a strong T_H2 cell response in infected individuals, the latter being of
657 unquestionable importance in host defense against parasites. Indeed, the actual contributions
658 of non-specific *vs*. specific IgE antibodies in host defense and parasite clearance are still
659 unclear and numerous experimental studies aiming at addressing this question have led to
660 different, sometimes opposing, conclusions (recently reviewed in (Mukai, Tsai et al. 2016)).
661 Also, protective *vs*. detrimental roles of IgE antibodies in anti-parasite immunity appear to be
662 parasite-dependent. For instance, data from experiments with IgE-deficient mice indicate
663 beneficial functions for IgE in models of *Trichinella sprialis* (Gurish, Bryce et al. 2004),
664 *Schistosoma mansoni* (King et al. 1997), *Brugia Malayi* (Spencer et al. 2003),
665 *Nippostrongylus brasiliensis* and *Heligmosomoides polygyrus* (Schwartz, Turqueti-Neves et
666 al. 2014). On the other hand, experiments with IgE- or FcεRIα-deficient mice in other studies
667 showed no effect or *decreased* parasite burden in infections with *H. polygyrus* (McCoy, Stoel
668 et al. 2008), *Strongyloides venezuelensis* (Matsumoto, Sasaki et al. 2013) or *S. mansoni*
669 (Jankovic, Kullberg et al. 1997). Among the factors potentially contributing to these
670 discrepancies, one could cite differences in experimental approaches (transgenic [IgE- or
671 FcεRIα-deficient mice] or pharmacological [anti-IgE treatments]), the experimental model
672 and/or the genetic background of the mice (Mukai, Tsai et al. 2016).
673

674 *4.2.2. Host defense against venoms*

675

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

684

685 Recently, Profet's hypothesis was supported by experimental evidence demonstrating
686 that IgE antibodies could contribute to acquired resistance against honeybee and snake
687 venoms (Marichal, Starkl et al. 2013, Palm, Rosenstein et al. 2013, Starkl, Marichal et al.
688 2016). Marichal, Starkl *et al*. characterized the immune response of mice following
689 subcutaneous injection of whole bee venom to mimic bee stings (Marichal, Starkl et al. 2013).
690 The venom induced a robust adaptive type 2 immune response associated with development
691 of venom-specific T_H2 cells and IgE, and this acquired immune response was associated with
692 increased resistance of mice (quantified by survival and body temperature) against a
693 subsequent challenge with bee venom. Experiments involving passive immunization and
694 transgenic animals deficient in IgE or FcεRI demonstrated that IgE antibodies and IgE
695 effector mechanisms played a crucial role in mediating acquired host resistance against bee
696 venom (Marichal, Starkl et al. 2013). In a complementary study, Palm, Rosenstein *et al*.
697 provided experimental evidence that a type 2 immune response directed against the bee
698 venom component phospholipase A2 (PLA2) was able to confer protection against a

699 subsequent near lethal dose of PLA2, and that such protection was dependent on FcεRI (Palm,
700 Rosenstein et al. 2013). Subsequently, Starkl, Marichal *et al*. found that IgE effector
701 mechanisms also played a critical role in acquired host defense against the venom of the
702 Russell's viper (Starkl, Marichal et al. 2016).
703
704 The strong evidence for the important protective function of IgE and IgE effector cells
705 in immune defense against venoms in mice challenges the current view of the function of IgE
706 in (venom-) allergic humans (Artis, Maizels et al. 2012). Therefore, future investigations are
707 needed to determine whether IgE-associated responses can enhance resistance to other toxins,
708 and to understand why, in some species or individuals, exposure to the same venom or venom
709 component may induce either a protective IgE-dependent adaptive immune response, as in the
710 mouse studies described above (Marichal, Starkl et al. 2013, Palm, Rosenstein et al. 2013,
711 Starkl, Marichal et al. 2016), or a deleterious and potentially fatal allergic reaction (*i.e.*,
712 anaphylaxis) (Saelinger and Higginbotham 1974, Charavejasarn, Reisman et al. 1975). This
713 question is of great interest and relevance for basic and clinical allergy research.
714
715 # 5. Targeted anti-IgE therapies
716
717 ## 5.1 Anti-IgE antibodies
718
719 *5.1.1. Omalizumab*
720
721 Omalizumab is a recombinant humanized IgG1 monoclonal antibody directed against
722 human IgE sold by Novartis and Genentech under the trade name Xolair® (Presta, Lahr et al.
723 1993). It binds to the Cε3 domain of free IgE, and thereby impairs binding of IgE to both

724 FcεRI and CD23 (Chang, Davis et al. 1990, Selb, Eckl-Dorna et al. 2016, Davies, Allan et al.
725 2017) (**Figure 5**). Importantly, omalizumab does not recognize IgE already bound to FcεRI or
726 CD23, and therefore cannot induce cell activation by crosslinking of IgE receptors (Chang,
727 Davis et al. 1990, Davies, Allan et al. 2017).
728
729 The IgE binding site of omalizumab has been characterized recently by molecular
730 modeling and crystallography (Zheng, Li et al. 2008, Wright, Chu et al. 2015, Pennington,
731 Tarchevskaya et al. 2016, Davies, Allan et al. 2017). Omalizumab binds to symmetric sites on
732 the two IgE Cε3 domains: it does not directly mask the FcεRI binding site on IgE, but rather
733 induces major conformational changes in the Cε3 domains that inhibit interaction with FcεRI
734 (Zheng, Li et al. 2008, Wright, Chu et al. 2015, Pennington, Tarchevskaya et al. 2016, Davies,
735 Allan et al. 2017). Davies and colleagues reported that, furthermore, IgE binding to CD23 is
736 sterically hindered by Omalizumab due to overlapping binding sites on each Cε3 domain
737 (Davies, Allan et al. 2017). While omalizumab is alleged to be unable to bind IgE already
738 bound to FcεRI, *in vitro* data suggest that omalizumab could also facilitate the dissociation of
739 FcεRI-bound IgE (Eggel, Baravalle et al. 2014).
740
741 The first randomized, double blind, placebo controlled trials were conducted in 1996
742 to assess the tolerability and efficiency of omalizumab in patients with allergic asthma
743 (Boulet, Chapman et al. 1997, Fahy, Fleming et al. 1997). These trials showed a reduction of
744 free serum IgE levels (but an increase in total serum IgE, *i.e.* free IgE and IgE complexed
745 with omalizumab), and improved responses to inhaled allergens following omalizumab
746 therapy ((Boulet, Chapman et al. 1997, Fahy, Fleming et al. 1997). In addition to the
747 reduction of free serum IgE levels, treatment with omalizumab also induced a decrease in the
748 expression of FcεRI on the surface of basophils, DCs and mast cells (Saini, MacGlashan et al.

749 1999, Prussin, Griffith et al. 2003, Lin, Boesel et al. 2004). In 2003, Xolair® was approved
750 for the treatment of moderate to severe persistent allergic asthma, and is now also approved
751 for the treatment of chronic spontaneous urticaria (CSU) (Maurer, Rosen et al. 2013, Chang,
752 Chen et al. 2015, Zhao, Ji et al. 2016). In addition, more than 150 clinical trials of
753 omalizumab are now listed on the website clinicaltrials.gov, in various diseases including
754 food and venom allergies (in combination with allergen-specific immunotherapy), allergic
755 rhinitis or mastocytosis. It is, however, important to note that, although Xolair® is generally
756 well tolerated, it can induce side effects ranging from skin inflammation (at the site of
757 subcutaneous injection) to systemic anaphylaxis (in 0.1-0.2% of patients) (Harrison, MacRae
758 et al. 2015, Lieberman, Umetsu et al. 2016).
759
760 *5.1.2. Ligelizumab*
761
762 Ligelizumab (QGE031) is a more recent humanized anti-IgE antibody developed by
763 Novartis. It is also directed against Cε3, but is designed to achieve improved IgE suppression,
764 with an equilibrium dissociation constant (K_D) of 139 pM (as compared to the K_D of
765 omalizumab, ~6-8nm) (Arm, Bottoli et al. 2014) (**Figure 5**). The first clinical results of
766 ligelizumab treatment indicated that this antibody can reduce free-IgE and basophil FcεRI
767 with an efficiency superior to that of omalizumab (NCT01716754). Although the authors did
768 not observe serious adverse events in this study, one patient treated with ligelizumab
769 developed systemic symptoms (Arm, Bottoli et al. 2014). In 2016, ligelizumab was tested in
770 patients with mild allergic asthma, and was shown to have greater efficacy than omalizumab
771 on inhaled and skin allergen responses in these patients (NCT01703312) (Gauvreau, Arm et
772 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, Maciuca 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

797 XmAb7195 is a monoclonal anti-IgE antibody developed by Xencor through
798 humanization, affinity maturation, and Fc engineering of the murine parental antibody of
799 omalizumab (MaE11) (Chu, Horton et al. 2012). XmAb7195 has an IgE-binding affinity 5.3-
800 fold higher than that of omalizumab. In addition, two point mutations in the IgG1 Fc portion
801 of the mAb (G236R and L328R) increase the binding affinity to inhibitory IgG receptor
802 FcγRIIB by 400 times compared to omalizumab (Chu, Horton et al. 2012). The authors
803 demonstrated that XmAb7195 could block free IgE and inhibit IgE production in B cells
804 through co-engagement of mIgE and FcγRIIB (Chu, Horton et al. 2012) (**Figure 5**). In a first-
805 in-human phase 1a trial in healthy volunteers (NCT02148744), XmAb7195 decreased IgE
806 levels below the limit of detection in 90% of subjects that had detectable IgE levels at
807 baseline. Transient thrombocytopenia was observed at a dose of 3.0 mg/kg, but no other
808 major adverse events were reported (Gershman, Goldwater et al. 2016). A phase 1b study on
809 the safety, tolerability and bioavailability of a subcutaneous formulation of XmAb7195 has
810 been recently completed (NCT02881853), but the results of this study have not yet been
811 reported.
812
813 *5.1.5. MEDI4212*
814
815 MEDI4212 is a human IgG1 anti-IgE antibody developed by MedImmune. MEDI4212
816 was generated using phage display technology, combined with targeted mutagenesis of V_H
817 and V_L sequences to increase its affinity for IgE (Cohen, Dobson et al. 2014). Like
818 omalizumab, MEDI4212 does not recognize IgE already bound to FcεRI, but the authors
819 report that MEDI4212 binds free IgE with an affinity of 1.95 pM, more than 100-fold higher
820 than omalizumab (Cohen, Dobson et al. 2014) (**Figure 5**). Analysis of the crystal structure of
821 IgE Cε3-4 domains in complex with MEDI4212 Fab portion revealed that MEDI4212

822 recognizes residues in the Cε3 and Cε4 domains, and targets critical residues in Cε3 also
823 involved in binding to FcεRI. This suggests that MEDI4212 directly competes with FcεRI for
824 IgE binding (Cohen, Dobson et al. 2014).
825
826 Since MEDI4212 recognizes residues in the IgE Cε3-4 domains, it can also bind mIgE
827 on the surface of B cells. MEDI4212 was further engineered in order to increase its potential
828 to eliminate IgE-expressing B cells through antibody-dependent cell-mediated cytotoxicity
829 (ADCC) (Nyborg, Zacco et al. 2016). The authors chose to insert mutations in the Fc portion
830 of MEDI4212 in order to improve its affinity for the IgG receptor FcγRIIIA, as ADCC can be
831 performed by natural killer (NK) cells that express FcγRIIIA. Indeed, *in vitro* experiments
832 revealed that, thus Fc-engineered, MEDI4212 could eliminate class-switched human IgE B
833 cells more efficiently (Nyborg, Zacco et al. 2016). A phase I study on the pharmacokinetics,
834 pharmacodynamics, and safety of MEDI4212 in subjects with atopy was initiated in 2012
835 (NCT01544348); and demonstrated that MEDI4212 rapidly reduced free IgE to a greater
836 extent than omalizumab. However, recovery of free IgE to baseline was much faster in
837 patients receiving MEDI4212 as compared as omalizumab, which was attributed to a rapid
838 decrease of serum MEDI4212. Since then, no other study has been initiated with this
839 antibody.
840
841 **5.2. Anti-IgE, anti-FcεRI and anti-CD23 DARPins**
842
843 Designed ankyrin repeat proteins (DARPins) are engineered small proteins that can
844 recognize targets with high specificity and with affinity in the low nanomolar range (Binz,
845 Amstutz et al. 2004, Pluckthun 2015). In 2009, Eggel and collaborators reported identification
846 of two monovalent DARPins, termed B-A4-85 and C-A3-30, displaying high affinity for two

847 different epitopes on human FcεRIα (Eggel, Baumann et al. 2009). They further produced a
848 bispecific anti-FcεRIα DARPin (designated 30/85) by linking sequences of the two
849 monovalent DARPins with a $[Gly_4–Ser]_4$ linker. Remarkably, this bispecific DARPin showed
850 greater affinity than IgE for FcεRIα, and was able to inhibit IgE-FcεRIα interaction and IgE-
851 mediated degranulation of rat basophilic leukemia cells expressing human FcεRIα (RBL-
852 2H3-huα cells), with an effect similar to that of omalizumab (Eggel, Baumann et al. 2009)
853 (**Figure 5**).
854
855 Using a similar strategy, the same group reported identification of several DARPins
856 binding human IgE (**Figure 5**). Among these, the DARPins E2_79 and E3_54 were able to
857 inhibit binding of IgE to either FcεRIα or omalizumab, and inhibit IgE-mediated activation of
858 RBL-2H3-huα cells with higher efficacy than omalizumab (Baumann, Eggel et al. 2010). It
859 was further demonstrated that E2_79 not only prevented binding of free IgE to FcεRI, but also
860 actively disrupted pre-formed IgE:FcεRI complexes (Kim, Eggel et al. 2012). Such facilitated
861 IgE dissociation was observed both *in vitro*, *ex vivo* in primary human basophils, and *in vivo*
862 in human FcεRI transgenic mice (Kim, Eggel et al. 2012, Eggel, Baravalle et al. 2014),
863 suggesting that anti-IgE DARPins might be suitable drug candidates to desensitize allergic
864 patients.
865
866 Another DARPin (E3_53) can recognize both free IgE and IgE bound to FcεRI. This
867 DARPin was linked to the Fc portion of human IgG1 (using a $[Gly_4–Ser]_3$ linker) to produce a
868 fusion protein capable of cross-linking FcεRI-bound IgE with the inhibitory receptor
869 FcγRIIB. This molecule, termed DE53-Fc, was able to reduce allergen-induced basophil
870 activation *ex vivo* using whole blood samples from allergic patients (Eggel, Buschor et al.
871 2011). Furthermore, by using blocking antibodies against FcγRIIB, the authors demonstrated

that binding of DE53-Fc to FcγRIIB 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 FcγRIIB 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 FcγRIIB, it is still ambiguous whether human mast cells also express this inhibitory receptor (Zhao, Kepley et al. 2006). Therefore, whether cross-linking of FcεRI-bound IgE to FcγRIIB 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. Fcε-Fcγ fusion proteins

The human Fcγ-Fcε bifunctional fusion protein consists of the Fc region of human IgG1 (hinge-Cγ2-3) linked to the Fc portion of human IgE (Cε2-4) by a 15 amino acid linker $(Gly_4Ser)_3$ (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 FcεRI, and could thereby be used to 'desensitize' mast cells and basophils (**Figure 5**). It could also bind to IgG FcγRs through its Cγ2-3 domains, and it was therefore proposed that GE2 could block IgE-mediated mast cell and basophil activation through co-engagement of FcεRI with the inhibitory receptor FcγRIIB (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

897 could also block IgE-mediated passive cutaneous anaphylaxis (PCA) in transgenic mice
898 expressing human FcεRI (Zhu, Kepley et al. 2002). In addition to its effect on mast cells and
899 basophils, it was proposed that the fusion protein could also inhibit allergic inflammation
900 through effects on FcεRI-expressing Langerhans cells (Kepley, Zhang et al. 2003), and inhibit
901 IgE class switch recombination in B cells by co-aggregating CD23 and FcγRII (Yamada, Zhu
902 et al. 2003). Several attempts were subsequently made to improve the efficiency of the fusion
903 protein, such as removal of the $(Gly_4Ser)_3$ linker, or mutations in the Cγ portion to improve
904 binding to FcγRIIB and/or decrease binding to FcγRIII (Allen, Kepley et al. 2007). However,
905 most of these modifications altered the effectiveness of the fusion protein to inhibit FcεRI-
906 mediated functions (Allen, Kepley et al. 2007). Nevertheless, and as described above (part
907 *5.2*), while basophils undoubtedly express FcγRIIB, it is still unclear whether human mast
908 cells express FcγRIIB *in vivo* (Zhao, Kepley et al. 2006).
909
910 The effects of GE2 were also tested in non-human primates. Rhesus monkeys have
911 been reported to exhibit skin test reactivity and serum IgE directed against dust mites
912 (Schelegle, Gershwin et al. 2001, Zhang, Kepley et al. 2004). Taking advantage of this, Zhang
913 and collaborators showed that GE2 was able to inhibit dust mite allergen-induced skin
914 reactivity in rhesus monkeys in a dose-dependent manner (Zhang, Kepley et al. 2004). In a
915 subsequent study, GE2 demonstrated efficacy in a model of house dust mite-induced allergic
916 asthma in cynomolgus monkeys (Van Scott, Mertsching et al. 2008). The effects of GE2
917 lasted for 4 weeks and were associated with reduced numbers of circulating basophils and
918 reduced FcεRI expression on basophils. However, repeated injections of GE2 induced the
919 production of serum antibodies against the fusion protein, and increased occurrence of serious
920 adverse events, including anaphylaxis (Van Scott, Mertsching et al. 2008).
921

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.

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951

952 ## 8. Conflict of Interest Statement

953

954 E.C. is an employee of Neovacs SA. L.L.R. reports serving as consultant for Neovacs SA. All
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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 Cε 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), FcεRI- and CD23-binding sites are indicated.

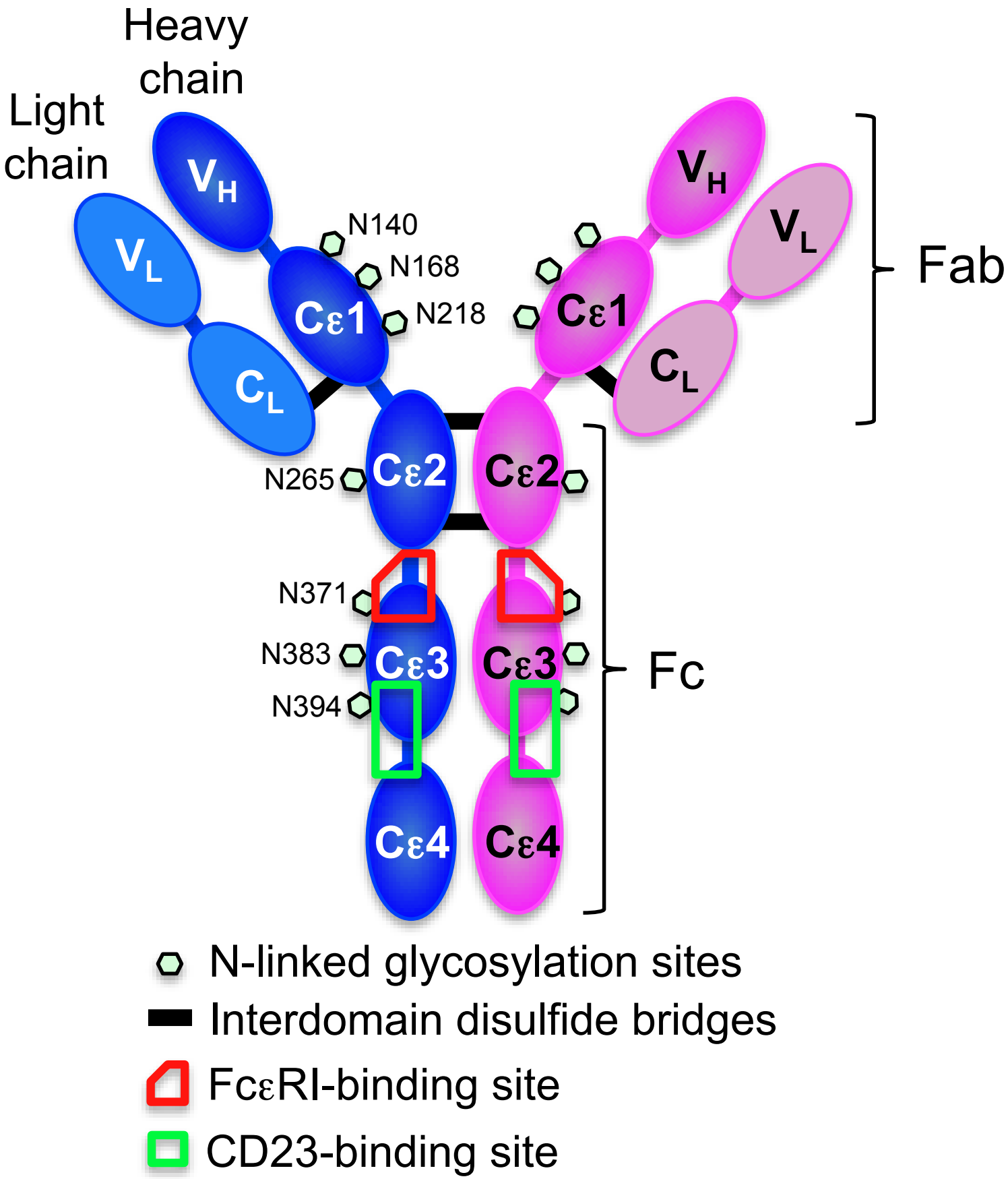
Figure 2. Structure of FcεRI and its interaction with IgE. a. FcεRI is expressed on mast cells and basophils as a tetramer formed with one α subunit, one β subunit and a dimer of disulfide-linked γ subunits. IgE binds the receptor via surface loops in Cε3, with contributions from the Cε2-Cε3 linker region. **b.** The two Cε3 domains of IgE bind distinct sites on FcεRIα, 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 FcεRI or CD23. The Cε3 domains of free IgE are found in a 'closed' conformation in which the FcεRIα binding site in Cε3 is masked (middle; Protein Data Bank [PDB] ID: 2WQR). Cε3 adopts an 'opened' conformation upon binding to FcεRI, which is incompatible with CD23 binding (left; PDB ID: 1F6A-2). By contrast, Cε3 adopts a 'closed' conformation upon binding to CD23, which is incompatible with FcεRI 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


Heavy chain
Light chain
V_H
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C_L
Cε1
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Cε2
N371
N383
Cε3
N394
Cε4
V_H
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Cε1
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Fab
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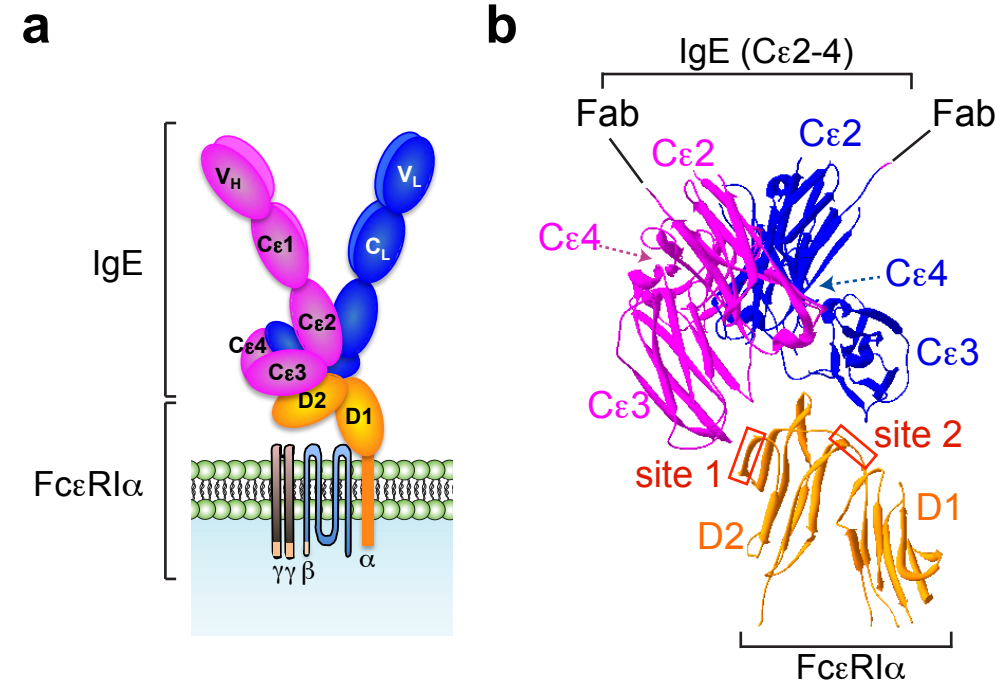
N-linked glycosylation sites
Interdomain disulfide bridges
FcεRI-binding site
CD23-binding site

Figure 2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IgE
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α
b
IgE (Cε2-4)
Fab
Fab
Cε2
Cε2
Cε4
Cε4
Cε3
Cε3
site 2
site 1
D1
D2
FcεRIα

Figure 3

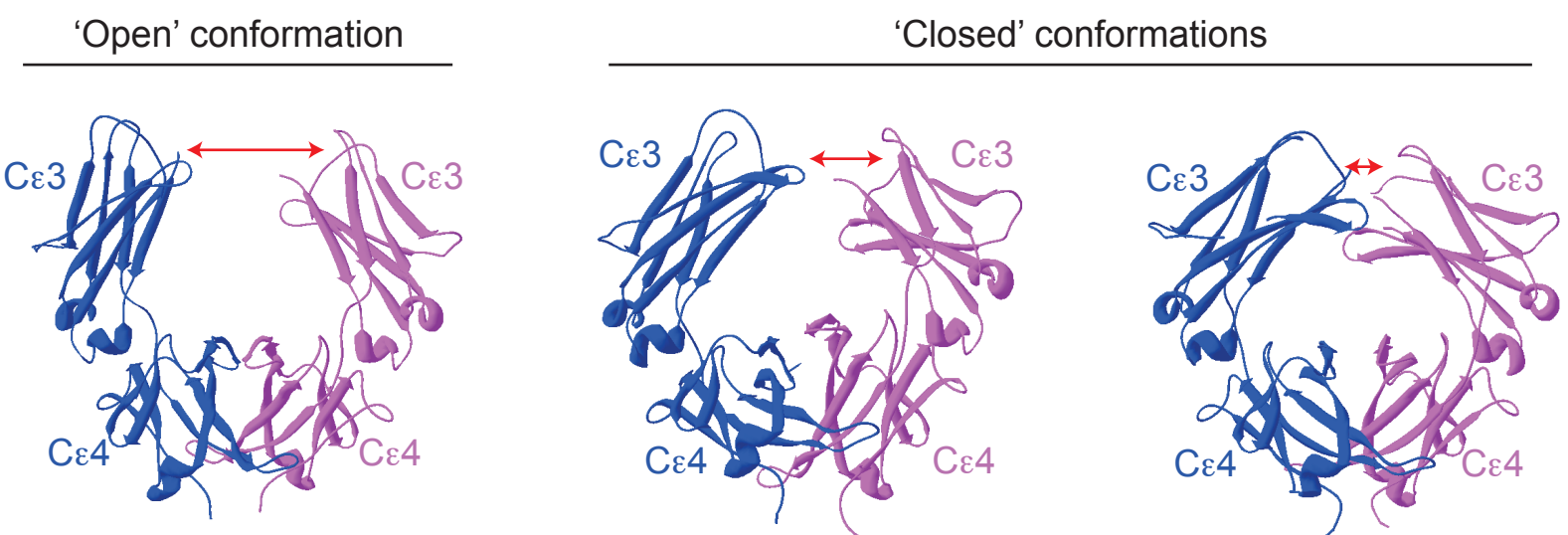

'Open' conformation
'Closed' conformations
Cε3
Cε3
Cε4
Cε4
Cε3
Cε3
Cε4
Cε4
Cε3
Cε3
Cε4
Cε4
FcεR1-bound IgE
Free IgE
CD23-bound IgE

Figure 4

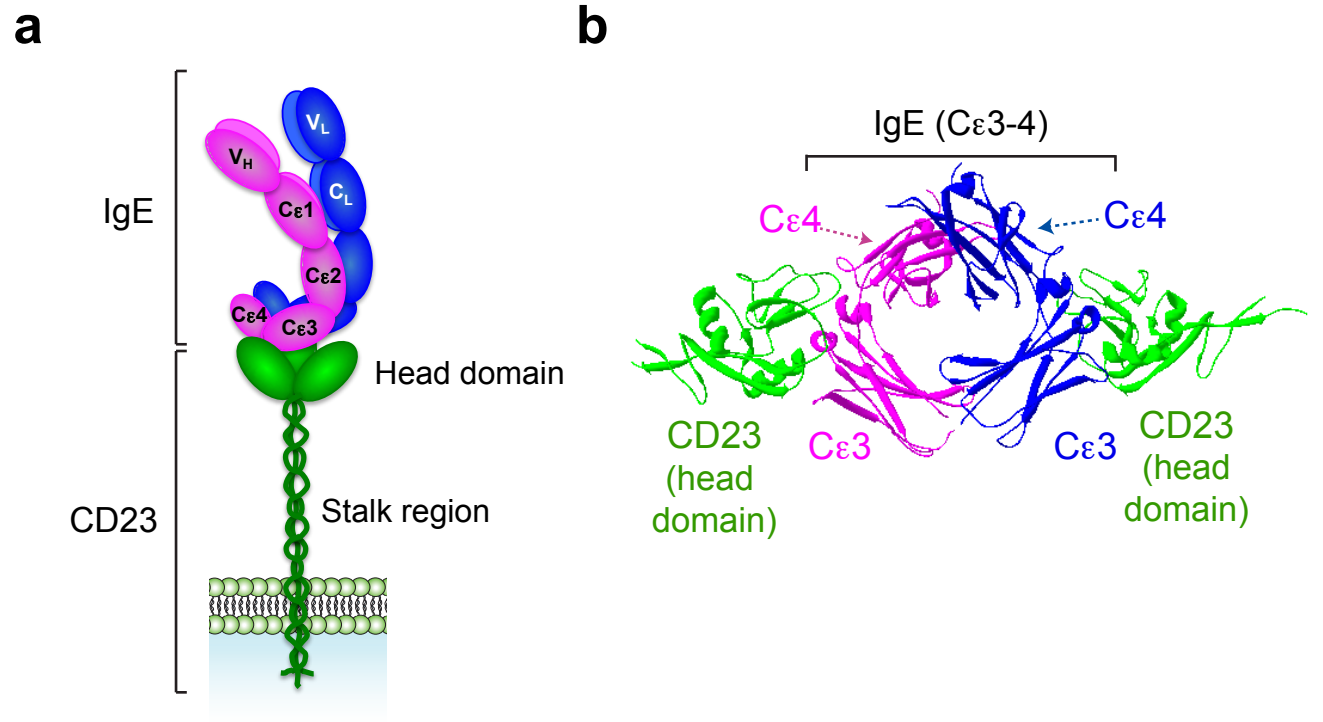
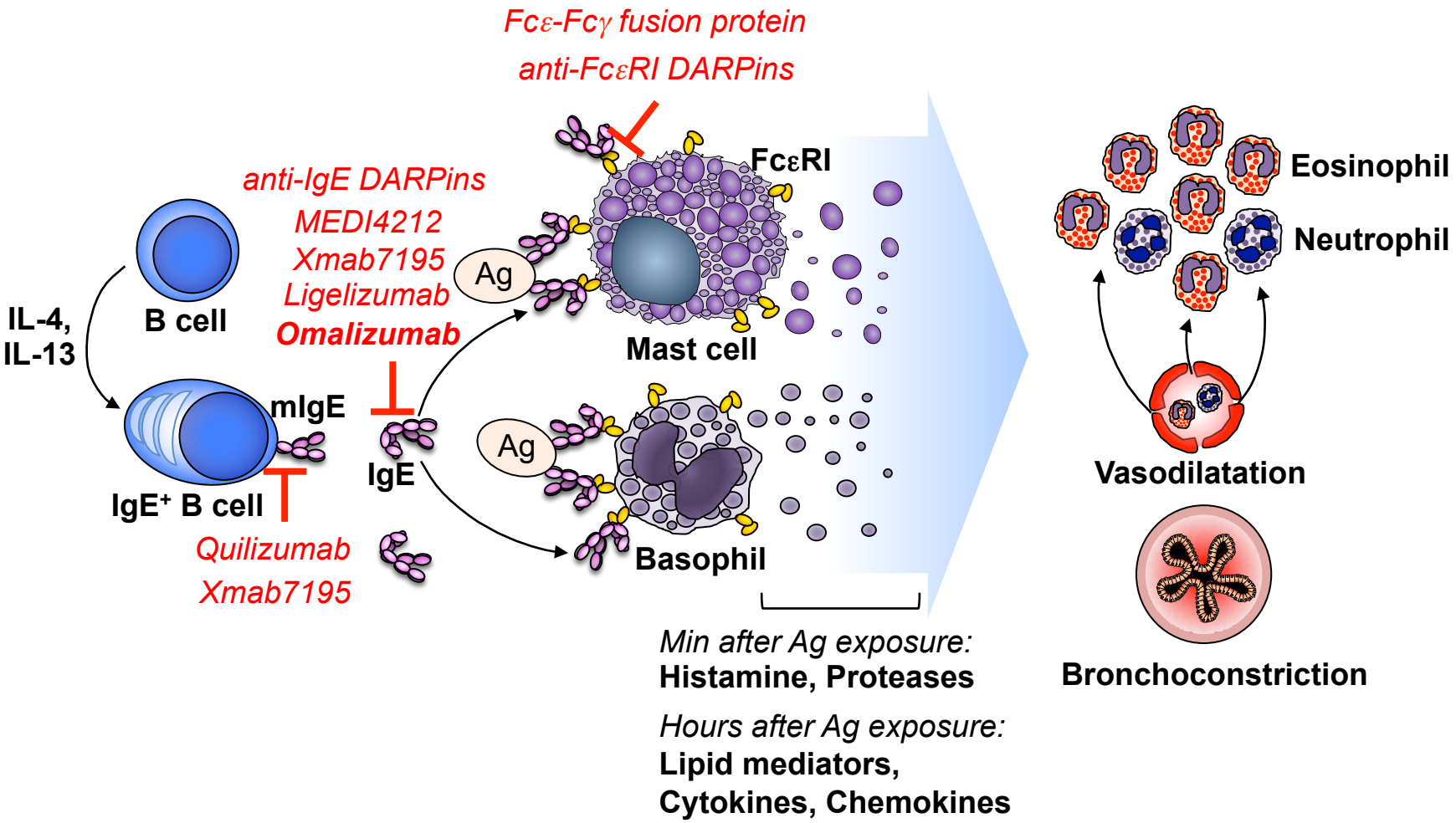

a
IgE
CD23
V_L
V_H
C_L
Cε1
Cε2
Cε4
Cε3
Head domain
Stalk region
b
IgE (Cε3-4)
Cε4
Cε4
CD23 (head domain)
Cε3
Cε3
CD23 (head domain)

Figure 5


Fcε-Fcγ fusion protein
anti-FcεRI DARPins
anti-IgE DARPins
MEDI4212
Xmab7195
Ligelizumab
Omalizumab
FcεRI
Ag
IL-4,
IL-13
B cell
mIgE
IgE
IgE+ B cell
Quilizumab
Xmab7195
Mast cell
Ag
Basophil
Min after Ag exposure:
Histamine, Proteases
Hours after Ag exposure:
Lipid mediators,
Cytokines, Chemokines
Eosinophil
Neutrophil
Vasodilatation
Bronchoconstriction