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# Approaches to target IgE antibodies in allergic diseases

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**24 Abstract**

25

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

35

36

37

**38 Keywords**

39 Immunoglobulin, FcεRI, FcεRII, CD23, omalizumab, DARPs, antibody, allergy,  
40 anaphylaxis, asthma, atopic dermatitis

41

42

43 **Abbreviations**

44

45 **AD**: atopic dermatitis; **Ag**: antigen; **ADAM10**: a disintegrin and metalloprotease 10; **ADCC**:  
46 antibody-dependent cell-mediated cytotoxicity; **AECs**: airway epithelial cells; **ASST**:  
47 autologous serum skin test; **C $\epsilon$** : constant epsilon domain of IgE; **C<sub>L</sub>**: constant region of an  
48 antibody's light chains; **CSU**: chronic spontaneous urticaria; **DARPs**: designed ankyrin  
49 repeat proteins; **DC**: dendritic cell; **Fab**: fragment antigen-binding region; **Fc**: fragment  
50 crystallizable region of an antibody; **HRF**: histamine releasing factor; **IECs**: intestinal  
51 epithelial cells; **Ig**: immunoglobulin; **IL**: interleukin; **ITAM**: immunoreceptor tyrosine-based  
52 activation motif; **mIgE**: membrane-bound IgE; **PCA**: passive cutaneous anaphylaxis; **PLA2**:  
53 phospholipase A2; **PSA**: passive systemic anaphylaxis; **Tg**: transgenic; **T<sub>H</sub>2**: T cell helper  
54 type 2; **TPO**: Thyroperoxidase; **V<sub>H</sub>**: variable region of an antibody's heavy chains; **V<sub>L</sub>**:  
55 variable region of an antibody's light chains; **WT**: wild type.

56

57

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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  $\gamma$ E 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  $\gamma$ E 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



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.

## 151 **2. IgE structure**

152

153 IgE antibodies are composed of two identical heavy chains (each comprising a  
154 variable  $V_H$  domain and four constant  $C\epsilon$  domains) and two identical light chains (composed  
155 of a variable  $V_L$  domain and a constant  $C_L$  domain) with a total molecular weight of 190 kDa  
156 (Gould and Sutton 2008, Wu and Zarrin 2014) (**Figure 1**). Similar to other antibody classes,  
157 the Fab region of IgE is responsible for antigen recognition and binding, while the effector  
158 function of IgE is determined by the carboxy-terminal Fc portion (Gould and Sutton 2008,  
159 Wu and Zarrin 2014). IgE shares a similar overall structure with IgG, with the exception of an  
160 additional domain in the heavy chain ( $C\epsilon_2$ ). As detailed in part 3.1.3, this additional  $C\epsilon_2$   
161 domain corresponds to the location of the flexible hinge region found in IgG, and plays a  
162 major role in enhancing the stability of the interaction between IgE and its high affinity  
163 receptor Fc $\epsilon$ RI (McDonnell, Calvert et al. 2001). The Fc $\epsilon$ RI binding site is located in the  $C\epsilon_3$   
164 domain and in the  $C\epsilon_2$ - $C\epsilon_3$  linker region (Garman, Wurzburg et al. 2000) (described in more  
165 detail in part 3.1.3). The binding site to the low affinity IgE receptor CD23 is also primarily  
166 located within the  $C\epsilon_3$  domain, with contributions from the  $C\epsilon_4$  domain (described in more  
167 detail in part 3.2.3) (**Figure 1**). The crystal structure of the human  $C\epsilon_3$ - $C\epsilon_4$  domains revealed  
168 that, by rotating relatively to  $C\epsilon_4$ ,  $C\epsilon_3$  can adopt either 'open' or 'closed' conformations. This  
169 conformational flexibility regulates the binding of IgE to both Fc $\epsilon$ RI and CD23 (Garman,  
170 Wurzburg et al. 2000, Wurzburg, Garman et al. 2000). These features are discussed in more  
171 detail in part 3.1.3 & 3.2.3. Several intra- and inter-domain disulphide bridges control the  
172 structure and activity of IgE, which is also regulated by glycosylation at various sites (**Figure**  
173 **1**). In particular, disruption of the glycosylation site found in the  $C\epsilon_3$  domain at asparagine-  
174 394 (N394) in humans, and N384 in mouse, abrogates the binding of IgE to Fc $\epsilon$ RI,

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

177

### 178 **3. IgE receptors**

179

#### 180 **3.1. The high affinity IgE receptor FcεRI**

181

##### 182 *3.1.1. FcεRI structure and expression*

183

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

197

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

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

205

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

210

### 211 3.1.2. Fc $\epsilon$ RI functions

212

213 Fc $\epsilon$ RI plays a key role in mediating the biological functions of IgE *in vivo*, which is  
214 best exemplified by the fact that Fc $\epsilon$ RI-deficient mice are fully resistant to IgE-mediated  
215 passive cutaneous anaphylaxis (PCA) and passive systemic anaphylaxis (PSA) (Dombrowicz,  
216 Flamand et al. 1993). These findings are most likely attributable to the  $\alpha\beta\gamma 2$  Fc $\epsilon$ RI tetramer  
217 expressed on the surface of mast cells, since mast cell-deficient mice are also resistant to IgE-  
218 mediated PCA and PSA (Miyajima, Dombrowicz et al. 1997, Feyerabend, Weiser et al. 2011,  
219 Lilla, Chen et al. 2011). Studies using transgenic mice expressing the human Fc $\epsilon$ RI $\alpha$  chain  
220 under the control of its own promoter have also given significant insight into the functions of  
221 human Fc $\epsilon$ RI (Dombrowicz, Brini et al. 1996, Dombrowicz, Lin et al. 1998, Greer, Wu et al.  
222 2014). *hFc $\epsilon$ RI $\alpha$ <sup>Tg</sup>* mice (bred on a mouse Fc $\epsilon$ RI-deficient background) express a ‘humanized’  
223 Fc $\epsilon$ RI receptor with a similar cellular distribution as that found in humans (Dombrowicz,  
224 Brini et al. 1996, Dombrowicz, Lin et al. 1998, Mancardi, Iannascoli et al. 2008, Greer, Wu et

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

234

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

249 the mouse receptor, as mice deficient in Fc $\epsilon$ RI clear serum IgE to the same extent as WT mice  
250 (Cheng, Wang et al. 2010).

251

252 It has also been suggested that human peripheral blood DCs use the  $\alpha$ 2 Fc $\epsilon$ RI trimer  
253 for allergen uptake and presentation to naive T cells (Maurer, Fiebiger et al. 1996). Using  
254 transgenic mice expressing human Fc $\epsilon$ RI $\alpha$  under the dependency of the CD11c promoter, in  
255 an attempt to restrict expression to DCs, these authors found that hFc $\epsilon$ RI-expressing DCs can  
256 efficiently prime naive T cells for T<sub>H</sub>2 differentiation, and amplify antigen-specific T<sub>H</sub>2  
257 responses *in vivo* (Sallmann, Reininger et al. 2011).

258

### 259 3.1.3. Binding of IgE to Fc $\epsilon$ RI

260

261 Mutagenesis studies have helped define the Fc $\epsilon$ RI binding epitope on IgE.  
262 Schwarzbaum and colleagues generated a mutant form of mouse IgE with a deletion of 45  
263 amino acids in the carboxy end of C $\epsilon$ 3: this mutant IgE was unable to bind Fc $\epsilon$ RI  
264 (Schwarzbaum, Nissim et al. 1989). Nissim and collaborators produced several chimeric IgE  
265 containing the C $\epsilon$ 2, C $\epsilon$ 3 and C $\epsilon$ 4 domains of human IgE (hereafter named C $\epsilon$ 2-4), in which  
266 various domains were replaced by their murine counterparts. This work confirmed that the  
267 Fc $\epsilon$ RI binding site mapped to the C $\epsilon$ 3 domain of IgE (Nissim, Jouvin et al. 1991). In 2000,  
268 Garman *et al.* determined the crystal structure of the IgE C $\epsilon$ 3-4 dimer bound to the  
269 extracellular part of Fc $\epsilon$ RI $\alpha$  (Garman, Wurzburg et al. 2000). Analysis of this crystal  
270 structure confirmed that each of the two chains of the IgE C $\epsilon$ 3-4 dimer could bind the  
271 receptor using surface loops in C $\epsilon$ 3, and revealed contributions of the C $\epsilon$ 2-C $\epsilon$ 3 linker region  
272 (Garman, Wurzburg et al. 2000).

273

274 Analysis of the crystal structures of the extracellular portion of human FcεRIα alone  
275 (Garman, Kinet *et al.* 1998) or in complex with a dimeric Cε3-4 fragment (Garman,  
276 Wurzburg *et al.* 2000) have also provided invaluable insight into how IgE interacts with  
277 FcεRI. The extracellular part of FcεRIα is formed of two immunoglobulin domains of about  
278 85 amino acids each (D1 and D2), with a heavily bent D1-D2 interface forming an overall  
279 structure of an inverted V shape (Garman, Kinet *et al.* 1998, Garman, Kinet *et al.* 1999)  
280 (**Figure 2**). The two Cε3 domains of IgE bind distinct sites on FcεRIα, one site found in the  
281 D2 domain, and a second site formed by a cluster of four surface-exposed tryptophans in the  
282 D1-D2 interface (Garman, Wurzburg *et al.* 2000). The presence of these two binding sites  
283 explains the 1:1 stoichiometry of the IgE-FcεRIα complex, which is essential to ensure that  
284 receptor crosslinking and activation occurs only upon multivalent antigen binding to IgE  
285 (Garman, Wurzburg *et al.* 2000).

286

287 A unique feature of the FcεRI receptor, as compared to other Fc receptors, is the  
288 distinctly slow dissociation rate of the IgE-FcεRIα complex ( $k_{\text{off}} \approx 10^{-5} \text{ s}^{-1}$ ). This translates  
289 into a half-life of about two weeks for IgE bound to FcεRI (compared to only hours for IgG  
290 complexes bound to Fcγ receptors), and ensures that tissue mast cells and basophils remain  
291 saturated with IgE (Geha, Helm *et al.* 1985, McDonnell, Calvert *et al.* 2001). McDonnell and  
292 collaborators showed that full human IgE molecules and dimeric IgE fragments comprising  
293 the Cε2, Cε3 and Cε4 domains (Cε2-4) have identical kinetics of dissociation with FcεRIα,  
294 while Cε3-4 displays a markedly enhanced dissociation kinetic (~20-fold), indicating that  
295 Cε2 plays a major role in enhancing the stability of the IgE-FcεRIα complex (McDonnell,  
296 Calvert *et al.* 2001). More recently, Holdom *et al.* published the crystal structure of human  
297 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 (Wurzberg, Garman et al. 2000, Wan, Beavil et al. 2002, Wurzberg 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 (Wurzberg,  
306 Garman et al. 2000, Wan, Beavil et al. 2002, Wurzberg 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 (Wurzberg, 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),



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

335

### 336 3.2.2. *CD23 functions*

337

338 CD23 is expressed on the surface of B cells, where it serves as a negative regulator of  
339 IgE synthesis. Several publications show increased levels of IgE in mice deficient for CD23  
340 (Stief, Texido et al. 1994, Yu, Kosco-Vilbois et al. 1994, Haczku, Takeda et al. 2000, Riffo-  
341 Vasquez, Spina et al. 2000, Lewis, Rapsomaniki et al. 2004). Conversely, transgenic mice  
342 overexpressing CD23 in B (and T) cells have markedly reduced levels of circulating IgE after  
343 immunization (Payet, Woodward et al. 1999). The regulation of IgE production seems to  
344 require the oligomerization of CD23, since serum IgE levels are also increased in mice treated  
345 with an antibody that binds to the stalk region of CD23 and thus blocks receptor  
346 oligomerization (Kilmon, Ghirlando et al. 2001, Ford, Kilmon et al. 2006). It is possible that  
347 CD23 on B cells plays an additional role(s) in regulating serum IgE levels, independently of  
348 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<sub>H</sub>2 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

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

377

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

386

### 387 3.2.3. *Binding of IgE to CD23*

388

389         Early mutagenesis studies mapped the IgE binding site of CD23 to discontinuous  
390 epitopes between residues 160-287 in the C-terminal head domain (Bettler, Maier et al. 1989,  
391 Bettler, Texido et al. 1992). These mutagenesis studies also suggested that binding of IgE  
392 requires six out of eight extracellular cysteine residues of CD23, which are likely involved in  
393 the formation of intramolecular disulfide bridges (Bettler, Texido et al. 1992). The head  
394 domain of CD23 is involved in IgE binding, since its proteolytic cleavage by the house dust  
395 mite protease *Der p 1* abrogates binding (Schulz, Sutton et al. 1997). Nevertheless, one  
396 mutagenesis study suggested that the stalk region of CD23 is also involved in IgE binding  
397 (Chen, Ma et al. 2002); a finding that was recently confirmed, indicating that the IgE-CD23  
398 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 C $\epsilon$ 2-4  
403 domains, that CD23 recognizes a motif in the C $\epsilon$ 3 domain of IgE (Vercelli, Helm et al. 1989).  
404 This was confirmed in a study using chimeric IgE molecules in which the human C $\epsilon$ 3 domain  
405 was replaced by mouse C $\epsilon$ 3: 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 C $\epsilon$ 3 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 C $\epsilon$ 3-4 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 C $\epsilon$ 3 domains but with a contribution from C $\epsilon$ 4 (Dhaliwal, Yuan et al. 2012) (**Figure 4**).  
413 Although the binding sites for Fc $\epsilon$ RI and CD23 are at opposite ends of the C $\epsilon$ 3 domain,  
414 binding of the two receptors to IgE is mutually exclusive. Indeed, binding of IgE to CD23  
415 induces conformational changes in C $\epsilon$ 3, leading to a highly ‘closed’ conformation  
416 incompatible with Fc $\epsilon$ RI binding (Borthakur, Hibbert et al. 2012, Dhaliwal, Yuan et al. 2012).  
417 Similarly, the ‘opened’ conformation adopted by C $\epsilon$ 3 upon binding to Fc $\epsilon$ RI 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 C $\epsilon$ 2 domain also contributes to CD23 binding, in addition to the  
421 known contributions of the C $\epsilon$ 3 and C $\epsilon$ 4 domains (Dhaliwal, Pang et al. 2017).

422

423 **3.3. Other IgE or Fc $\epsilon$ RI binding molecules**

424

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

438

439 Takizawa and collaborators reported that IgE immune complexes can bind to the  
440 mouse IgG receptors Fc $\gamma$ RIIB and Fc $\gamma$ RIII expressed on mast cells and macrophages, with an  
441 affinity similar to that of IgG immune complexes (Takizawa, Adamczewski et al. 1992). They  
442 further demonstrated that such binding to Fc $\gamma$ Rs can induce mast cell activation independently  
443 of Fc $\epsilon$ RI (Takizawa, Adamczewski et al. 1992). IgE immune complexes were also found to  
444 bind and activate mouse Fc $\gamma$ RIV, expressed on monocytes, macrophages and neutrophils  
445 (Hirano, Davis et al. 2007, Mancardi, Iannascoli et al. 2008). Confirming that Fc $\gamma$ RIV can act  
446 as a low-affinity receptor for mouse IgE, treatment of mice with an anti-Fc $\gamma$ RIV antibody  
447 inhibited late phase reactions in a model of IgE-mediated passive cutaneous allergic  
448 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

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

483

484 IgE-mediated anaphylaxis is abrogated in mice lacking the high affinity IgE receptor  
485 FcεRI (Dombrowicz, Flamand et al. 1993), as well as in mast cell-deficient mice  
486 (Feyerabend, Weiser et al. 2011, Lilla, Chen et al. 2011, Oka, Kalesnikoff et al. 2012),  
487 highlighting the importance of IgE-mediated mast cell activation in this reaction. Mast cells  
488 likely also play a key role in human anaphylaxis. Indeed, elevated levels of the mast cell  
489 specific protease tryptase have been detected during anaphylactic reactions in humans  
490 (Schwartz, Metcalfe et al. 1987, Schwartz 2006, Brown, Stone et al. 2013). Moreover, an  
491 increased incidence of anaphylaxis was reported in patients with mastocytosis, a disease  
492 characterized by increased numbers of mast cells (Schuch and Brockow 2017). By contrast,  
493 the role of basophils in anaphylaxis is more debated. So-called “Basophil activation tests” are  
494 used to confirm allergen sensitization in human patients. In these tests, which are performed  
495 on blood samples *ex vivo*, IgE-mediated activation of basophils is monitored by measuring  
496 up-regulation of surface markers such as CD63 and CD203c (Santos, Du Toit et al. 2015,  
497 Kim, Kim et al. 2016, Giavina-Bianchi, Galvao et al. 2017). Recently, Korosec and  
498 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 FcR $\gamma$  (a signaling subunit shared by Fc $\epsilon$ RI  
513 and activating IgG Fc $\gamma$  receptors), and was restored upon engraftment of these mice with  
514 basophils purified from WT mice but not from *FcR $\gamma$ <sup>-/-</sup>* 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 Fc $\epsilon$ RI 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<sub>H1</sub> and T<sub>H2</sub> skin responses, mast cell recruitment into draining lymph nodes and  
575 IgE production) were reduced in *FcεRI*<sup>-/-</sup> mice. In this model, T<sub>H2</sub> 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*<sup>-/-</sup> 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

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

625

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

636

## 637 **4.2. Protective roles of IgE**

638

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

643

### 644 *4.2.1. Host defense against parasites*

645

646 Helminth infections are generally associated with a "type 2" immune response,  
647 characterized by helper type 2 T (T<sub>H</sub>2) cells that typically produce IL-4, IL-5 and IL-13,  
648 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 Grecis, 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 spiralis* (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<sub>H</sub>2 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 PLA<sub>2</sub>, 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](http://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

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

775

### 776 5.1.3. *Quilizumab*

777

778 Quilizumab (MEMP1972A) is a humanized monoclonal antibody developed by  
779 Genentech targeting the M1' epitope which is present on membrane IgE (mIgE) but not on  
780 serum IgE (**Figure 5**). Brightbill and colleagues demonstrated, using genetically modified  
781 mice that contained the human M1' domain inserted into the mouse IgE locus, that quilizumab  
782 could reduce serum IgE and deplete IgE-producing plasma cells *in vivo*, without affecting  
783 other immunoglobulin isotypes (Brightbill, Jeet et al. 2010). Quilizumab has been tested in  
784 clinical trials in patients with allergic rhinitis (NCT01160861) and mild allergic asthma  
785 (NCT01196039) (Gauvreau, Harris et al. 2014). In both studies, reductions in total and  
786 allergen-specific serum IgE were observed, as well as improved clinical responses to allergen,  
787 suggesting that targeting mIgE can reduce IgE production in humans (Gauvreau, Harris et al.  
788 2014). In a subsequent trial (NCT01582503), treatment with quilizumab also reduced total  
789 and allergen-specific IgE in patients with allergic asthma uncontrolled by standard therapy.  
790 However, treatment with quilizumab had no impact on asthma exacerbations, lung functions,  
791 or patient-reported symptoms in this trial (Harris, Maciuca et al. 2016). Similarly, quilizumab  
792 reduced IgE levels by about 30% in CSU patients, but it did not lead to clinical improvements  
793 in patient's self-reported itch-severity scores (NCT01987947) (Harris, Cabanski et al. 2016).

794

### 795 5.1.4. *XmAb7195*

796

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 Fc $\gamma$ RIIB 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 Fc $\gamma$ RIIB (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<sub>H</sub>  
817 and V<sub>L</sub> sequences to increase its affinity for IgE (Cohen, Dobson et al. 2014). Like  
818 omalizumab, MEDI4212 does not recognize IgE already bound to Fc $\epsilon$ RI, 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 C $\epsilon$ 3-4 domains in complex with MEDI4212 Fab portion revealed that MEDI4212

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

825

826         Since MEDI4212 recognizes residues in the IgE C $\epsilon$ 3-4 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 Fc $\gamma$ RIIIA, as ADCC can be  
831 performed by natural killer (NK) cells that express Fc $\gamma$ RIIIA. 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-Fc $\epsilon$ RI 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<sub>4</sub>-Ser]<sub>4</sub> 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<sub>4</sub>-Ser]<sub>3</sub> 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

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

880

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

885

### 886 **5.3. Fc $\epsilon$ -Fc $\gamma$ fusion proteins**

887

888 The human Fc $\gamma$ -Fc $\epsilon$  bifunctional fusion protein consists of the Fc region of human  
889 IgG1 (hinge-C $\gamma$ 2-3) linked to the Fc portion of human IgE (C $\epsilon$ 2-4) by a 15 amino acid linker  
890 (Gly<sub>4</sub>Ser)<sub>3</sub> (Zhu, Kepley et al. 2002). As first described by Zhu *et al.*, this fusion protein  
891 (called GE2) was able to compete with IgE for the binding to Fc $\epsilon$ RI, and could thereby be  
892 used to ‘desensitize’ mast cells and basophils (**Figure 5**). It could also bind to IgG Fc $\gamma$ Rs  
893 through its C $\gamma$ 2-3 domains, and it was therefore proposed that GE2 could block IgE-mediated  
894 mast cell and basophil activation through co-engagement of Fc $\epsilon$ RI with the inhibitory  
895 receptor Fc $\gamma$ RIIB (Zhu, Kepley et al. 2002). Indeed, the authors demonstrated that GE2 was  
896 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<sub>4</sub>Ser)<sub>3</sub> linker, or mutations in the C<sub>γ</sub> 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



## 922 **6. Concluding remarks**

923

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

932

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

938

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940

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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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1669  
1670

1671 **Figure legends**

1672

1673 **Figure 1. IgE structure.** IgE antibodies consist of two identical heavy chains (composed of a  
1674 variable  $V_H$  domain and four constant  $C\epsilon$  domains) and two identical light chains (composed  
1675 of a variable  $V_L$  domain and a constant  $C_L$  domain). ‘Fab’: region responsible for antigen  
1676 recognition and binding. ‘Fc’: portion responsible for IgE effector functions. The positions of  
1677 interdomain disulfide bridges, N-linked glycosylation sites (in human IgE), Fc $\epsilon$ RI- and  
1678 CD23-binding sites are indicated.

1679

1680 **Figure 2. Structure of Fc $\epsilon$ RI and its interaction with IgE. a.** Fc $\epsilon$ RI is expressed on mast  
1681 cells and basophils as a tetramer formed with one  $\alpha$  subunit, one  $\beta$  subunit and a dimer of  
1682 disulfide-linked  $\gamma$  subunits. IgE binds the receptor via surface loops in  $C\epsilon 3$ , with contributions  
1683 from the  $C\epsilon 2$ - $C\epsilon 3$  linker region. **b.** The two  $C\epsilon 3$  domains of IgE bind distinct sites on  
1684 Fc $\epsilon$ RI $\alpha$ , one site found in the D2 domain (site 1), and a second site formed by a cluster of  
1685 four surface-exposed tryptophan residues in the D1-D2 interface (site 2) (Protein Data Bank  
1686 ID: 2Y7Q).

1687

1688 **Figure 3. Conformational changes in IgE Fc portion upon binding to Fc $\epsilon$ RI or CD23.**  
1689 The  $C\epsilon 3$  domains of free IgE are found in a ‘closed’ conformation in which the Fc $\epsilon$ RI $\alpha$   
1690 binding site in  $C\epsilon 3$  is masked (middle; Protein Data Bank [PDB] ID: 2WQR).  $C\epsilon 3$  adopts an  
1691 ‘opened’ conformation upon binding to Fc $\epsilon$ RI, which is incompatible with CD23 binding  
1692 (left; PDB ID: 1F6A-2). By contrast,  $C\epsilon 3$  adopts a ‘closed’ conformation upon binding to  
1693 CD23, which is incompatible with Fc $\epsilon$ RI binding (right; PDB ID: 4GKO).

1694

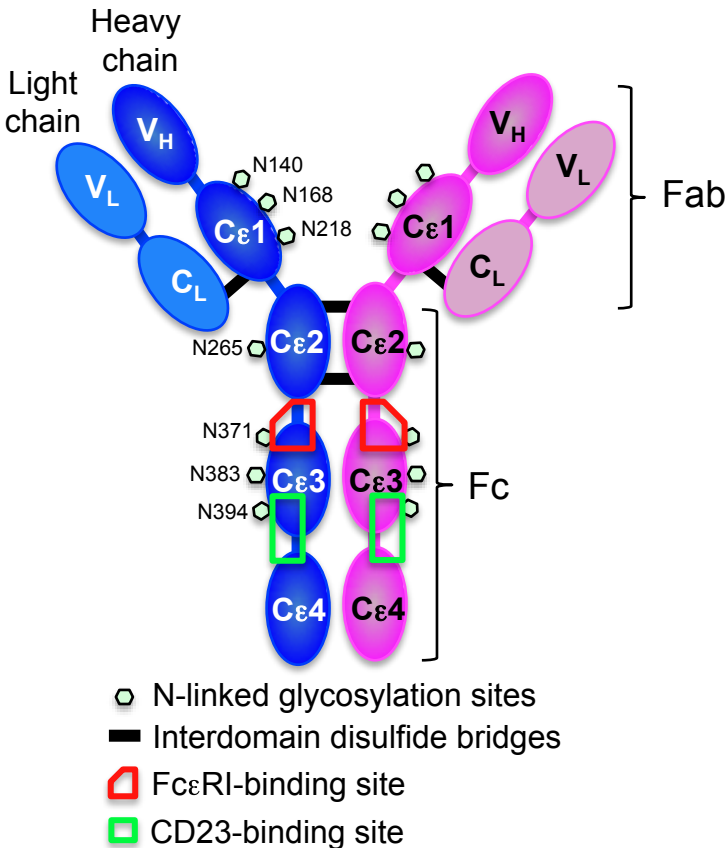
1695 **Figure 4. Structure of CD23 and its interaction with IgE. a.** CD23 self-associates as a  
1696 trimer, and is composed of an IgE-binding ‘head domain’ (which belongs to the C-type lectin  
1697 superfamily) linked to the membrane by an extracellular coiled-coil stalk region, and a small  
1698 cytoplasmic N-terminal domain. **b.** The IgE binding site of CD23 is located in the C-terminal  
1699 head domain (in green), with some additional contributions from the stalk region (not shown).  
1700 Two CD23 molecules bind to each IgE heavy chain, primarily to the C $\epsilon$ 3 domains but with a  
1701 contribution from C $\epsilon$ 4 (Protein Data Bank ID: 4GKO).

1702

1703 **Figure 5. Key role of IgE in allergic reactions.** Stimulation with the T<sub>H</sub>2 cytokines IL-4 and  
1704 IL-13 induces class-switching of B cells into IgE-producing cells. IgE binds to its high-  
1705 affinity receptor Fc $\epsilon$ RI on the surface of tissue mast cells and blood basophils. Upon exposure  
1706 to an allergen, in allergic patients, allergen recognition by allergen-specific IgE on the surface  
1707 of mast cells and basophils induces crosslinking of Fc $\epsilon$ RI, leading to degranulation and the  
1708 immediate release of histamine, proteases and other preformed mediators, as well as *de novo*  
1709 synthesis of lipid mediators (prostaglandins, leukotrienes,...), cytokines and chemokines.  
1710 These mediators can act locally or systemically, leading to the clinical features of immediate  
1711 hypersensitivity, such as bronchoconstriction, urticaria, diarrhea (when acting locally in the  
1712 airways, the skin and the gut, respectively) and vasodilatation. These mediators are also  
1713 responsible for late-phase allergic responses, entailing the recruitment of leukocytes, mainly  
1714 eosinophils and neutrophils. Several drugs have been developed to counteract the effects of  
1715 IgE. These drugs either target IgE production, block free IgE or compete with IgE for binding  
1716 to Fc $\epsilon$ RI. The only FDA-approved anti-IgE drug is Omalizumab, a humanized anti-IgE mAb  
1717 that blocks free IgE, and which is approved for the treatment of moderate to severe persistent  
1718 allergic asthma, and chronic spontaneous urticaria (CSU). Ag: antigen.

1719

# Figure 1

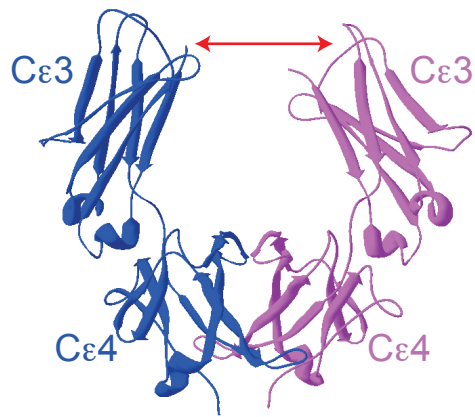




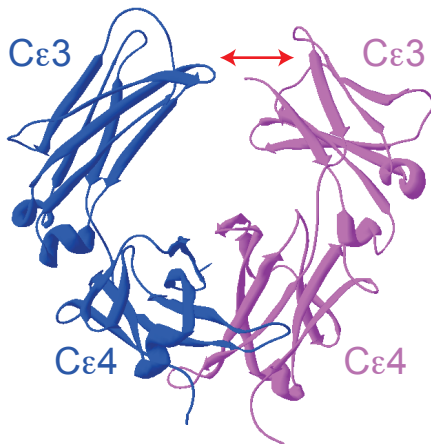
# Figure 3

'Open' conformation

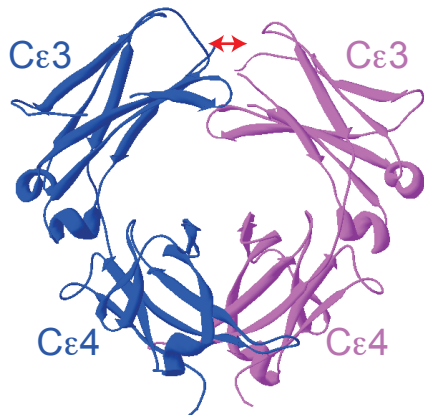
'Closed' conformations



FcεR1-bound IgE

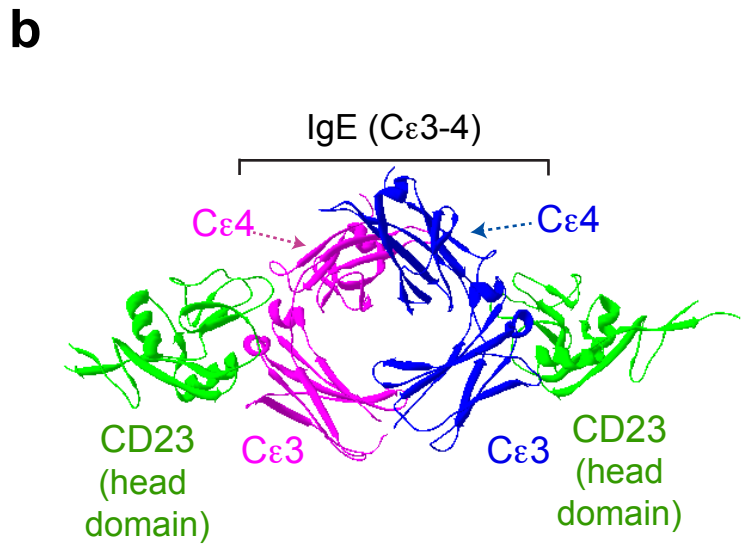
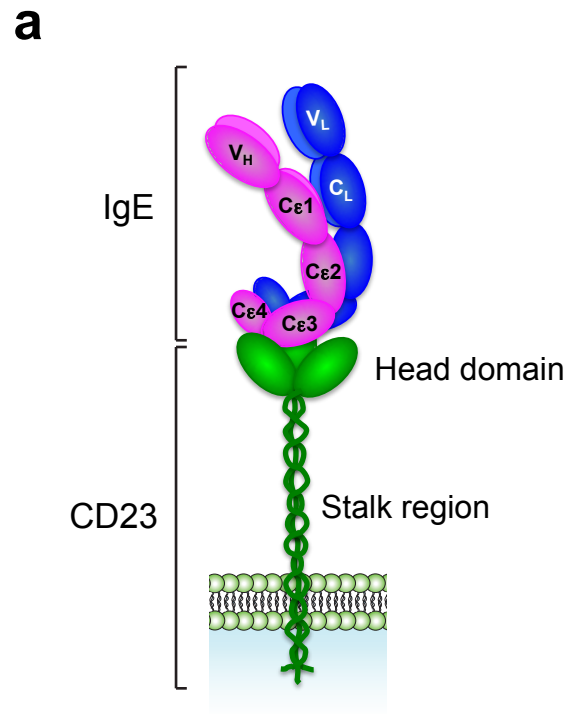


Free IgE



CD23-bound IgE

# Figure 4



# Figure 5

