Highlights:

- Summary of IgE, IgE receptor and anti-IgE crystal structures
- Comparison of anti-IgE treatment approaches and modes of action
- Classification of disruptive IgE inhibitors
- Suggestion of multi-level targeting concept using disruptive IgE inhibitors

Targeting IgE in allergic disease

Pascal Gasser[1,2] & Alexander Eggel[1,2]

[1]Department of BioMedical Research, University of Bern, Bern, Switzerland

[2]Department of Rheumatology, Immunology and Allergology, University Hospital Bern, Bern, Switzerland

Corresponding author: Alexander Eggel, Department of RIA, University of Bern, Switzerland. Phone: +41 31 632 22 87, E-mail: alexander.eggel@dbmr.unibe.ch.

Abstract

Immunoglobulin E (IgE) represents the least abundant antibody isotype in human serum. Nevertheless, it has the ability to induce remarkably potent allergic reactions. As a key component in the development and manifestation of hypersensitivity responses against usually non-hazardous foreign substances, IgE has become a major target of investigation and the subject of multiple therapeutic approaches for the treatment of allergies. Recent advances in the understanding of pathophysiologic mechanisms underlying IgE-associated allergic disorders have led to the generation of new drug candidates that are currently in development or under clinical evaluation. In this review, we highlight molecular and structural mechanisms underlying the different anti-IgE molecules and suggest a concept of multi-level targeting using a new class of disruptive IgE inhibitors to potentially optimize treatment efficacy.

Introduction

Since its discovery little more than 50 years ago, immunoglobulin E (IgE) has been attributed a wide variety of immunological functions including host defense against parasite infections and toxic venoms [1-3]. It has become increasingly evident that besides these beneficial properties IgE is a central player in the development and manifestation of allergic reactions [4]. Allergic rhinoconjunctivitis, atopic dermatitis, food allergies or allergic asthma are mostly IgE-dependent allergic conditions which manifest in symptoms ranging from mild local reactions to life-threatening systemic episodes. Generally, allergies are causing a marked reduction in quality of life and due to the low cost-effectiveness of targeted anti-IgE intervention strategies patients are often treated with unspecific therapeutics such as corticosteroids [5,6]. Recent advances in the understanding of basic molecular and structural properties of IgE and its receptors have helped to develop more targeted treatment approaches **(Table 1)** that we highlight in this review.

Structure-function relationship in IgE and its receptors

As a heterodimeric glycoprotein IgE consists of two light and two heavy chains. The heavy chain Fc-region of IgE (IgE-Fc) contains three consecutive Ig-domains, termed Cε2-4 **(Figure 1a)**. Compared to other immunoglobulins it lacks a flexible hinge region and thus adopts a rigid bent conformation in solution in which the Cε2 domain of both heavy chains asymmetrically fold back onto the Cε3 domain [7]. IgE exerts its effector functions through mutually exclusive interactions with its two principal cell surface receptors FcεRI and CD23 [8].

The high-affinity IgE receptor FcεRI is expressed as an $\alpha\beta\gamma_2$ heterotetramer primarily on human basophils and mast cells and as an $\alpha\gamma_2$ heterotrimer on human dendritic cells and monocytes [9]. FcεRIα binds IgE-Fc via two distinct interaction-sites [10].

While the Cε3 domains are in direct contact with the receptor, the Cε4 domains form a heavy chain dimerization interface. The 1:1 complex between IgE and FcεRIα is of remarkably high affinity (K_D~ 10^{-9}-10^{-10} M). Crystallization experiments have revealed that IgE undergoes a large conformational change upon FcεRIα binding in which the Cε3:Cε4 interdomain angle significantly increases **(Figure 1b)** [11]. This FcεRIα-bound IgE-Fc arrangement is referred to as open conformation **(Figure 1c)** [10,12]. Moreover, upon binding of FcεRIα the Cε2 domains increase their back-folding onto the Cε3 domain which further aggravates the asymmetrically bent conformation of IgE **(Figure 1d)** [11]. Interestingly, the Cε2 domains are not necessary for FcεRIα binding, but slow down both on and off rates for FcεRIα engagement [13]. Functionally, allergen induced cross-linking of FcεRI-bound IgE stimulates degranulation of basophils and mast cells that results in the release of pre-stored as well as *de novo* synthesized pro-inflammatory and vasoactive mediators inducing classical symptoms of an allergic disorder [14].

The second IgE cell surface receptor is CD23. Due to its carbohydrate binding head domain, it belongs to the C-type lectin superfamily [15]. Even though IgE is one of the most glycosylated mammalian immunoglobulins, binding to CD23 has been shown to be independent of lectin-glycan interactions [16,17]. Since monomeric IgE:CD23 complexes are typically unstable (K_D~ 10^{-6} - 10^{-7} M) CD23 is also referred to as low-affinity IgE receptor [18]. The presence of Ca^{2+} substantially enhances the affinity for IgE about 30-fold through the induction of conformational changes in the receptor [19,20]. Crystal structures have revealed that binding of IgE occurs via the CD23 head domain in a 2:1 stoichiometry **(Figure 1e)** [8,21]. A study using negative stain electron microscopy has recently described an additional contribution of the CD23 stalk region to IgE binding [22]. Given the asymmetric bent conformation of IgE, it has been reported that the two CD23 interaction sites have different binding kinetics and affinities

[21]. Upon CD23 binding the IgE-Fc adopts a closed conformation in which the Cε3-Cε4 interdomain angle significantly decreases **(Figure 1f)**. CD23 is mainly expressed on B-cells, epithelial cells as well as antigen-presenting cells and multiple studies have highlighted the role of CD23 in the regulation of IgE synthesis as well as allergen transport and presentation [23,24].

Classical inhibition of free serum IgE

Omalizumab, also known as Xolair®, is a humanized monoclonal anti-IgE antibody that has initially been approved for the treatment of moderate to severe persistent allergic asthma [25] including children ≥ 6 years of age [26]. More recently, it has been authorized for the use in patients with chronic spontaneous urticaria [27]. Additionally, off-label use of Omalizumab has revealed its efficacy in facilitating allergen updosing and desensitization in allergen-specific immunotherapy [28,29]. Omalizumab binds IgE with high affinity (K_D~ 7 x 10^{-9} M) [30]. Its primary mode of action is the neutralization and clearance of free serum IgE which further results in the destabilization and loss of FcεRI on mast cells and basophils [31]. Interestingly, treatment with Omalizumab has also been shown to reduce the number of circulating basophils [32]. Crystal structures of Omalizumab with a closed conformation IgE-G335C-Fc_{3-4} mutant helped to precisely map the binding-site of Omalizumab to the Cε3 domain of IgE **(Figure 1g)** and revealed that the inhibition of IgE binding to FcεRI is due to steric conflicts of the Omalizumab light-chain with FcεRIα, while there is barely any direct competition for FcεRIα binding residues [33]. An alternative possibility of Omalizumab-mediated inhibition of IgE:FcεRI complex formation has recently been suggested [34]. The authors of this study propose an allosteric mechanism in which Omalizumab binding induces an unbending of IgE that is associated with structural changes compromising FcεRI binding. Both studies agree that Omalizumab prevents binding of IgE to CD23,

which is dependent on direct competition for receptor-binding residues on IgE as well as major steric clashes between CD23 and Omalizumab [33]. Even though a recent study has reported that CD23 surface levels on B-cells of allergic patients correlate with allergen-specific IgE levels it remains elusive whether Omalizumab treatment has a direct effect on IgE-production in B-cells through inhibition of IgE:CD23 interaction [35].

QGE031, also known as Ligelizumab, is a humanized high-affinity anti-IgE antibody that is based on the previously developed CGP51901 antibody (i.e. Talizumab or TNX-901). Compared to Omalizumab, Ligelizumab binds IgE with significantly higher affinity (K_D~ 1.4 x 10^{-10} M) and suppresses IgE serum levels with six- to nine-fold higher potency [30]. Further, the reduction of cell surface IgE on circulating basophils is more sustained and the inhibition of skin prick responses to allergens is more pronounced upon Ligelizumab treatment. Despite promising results in a phase I study with mild allergic asthma patients (NCT01703312) [36], the phase 2 study with asthma patients (NCT02336425) has been discontinued. A phase 2b study, testing the efficacy and safety of Ligelizumab in patients with chronic spontaneous urticaria has recently been completed (NCT02477332) and results are pending.

Another anti-IgE antibody, called MEDI4212, has been engineered from a single-chain variable fragment selected against IgE using phage display [37]. It binds IgE with even higher affinity than Ligelizumab (K_D~ 2 x 10^{-12} M) and also inhibits binding to FcεRIα [37]. Further, MEDI4212 has been shown to inhibit IgE binding to CD23 on B-cells *in vitro*. Crystal structures of MEDI4212 in complex with IgE-Fc_{3-4} helped to map its interaction-site to the Cε3 domain of IgE and showed that it directly competes with FcεRIα but not CD23 binding residues on IgE **(Figure 1h)**. This study suggests that MEDI4212 locks IgE in an open conformation which is unable to bind CD23 [37]. In a Phase 1 clinical trial (NCT01544348) MEDI4212 showed superior results in

suppressing IgE levels compared to Omalizumab [38]. However, IgE levels returned to baseline faster in MEDI4212 treated patients, which might be due to its shorter serum half-life.

Targeting IgE producing B cells

Cross-linking of the B-cell receptor (BCR) without co-stimulation has been reported to induce apoptosis [39]. To exploit this mechanism and to test whether targeting and depletion of IgE producing B-cells might represent a suitable therapeutic strategy to decrease serum IgE levels an antibody specific for the membrane proximal domain (M1) of the IgE BCR has been developed [40]. This antibody, initially termed 47H4, successfully reduced the number of IgE expressing B-cells and decreased serum IgE levels in mice. Since afucosylation of antibodies increases their affinity to FcyRIIIA on NK-cells and thereby enhances the potentcy of antibody dependent cellular cytotoxicity (ADCC) a humanized, afucosylted version of 47H4, called Quilizumab, has been generated and tested in humans with allergic conditions. Quilizumab treatment of patients with allergic rhinitis and mild allergic asthma reduced baseline allergen-specific and total IgE in serum up to 30% in a phase 1b (NCT01160861) and 2a (NCT01196039) clinical trial [41]. These reductions were sustained for at least 6 months. While a significant amelioration of the allergen-induced symptoms in the early-asthmatic response to airway challenge was observed, no improvement was apparent for the late asthmatic response. In another phase 2 study in adults with inadequately controlled asthma (NCT01582503) no clinical significant improvement was achieved upon Quilizumab treatment [42]. Moreover, an additional phase 2 study in adults with refractory chronic spontaneous urticaria (NCT01987947) failed to demonstrate significant clinical efficacy of Quilizumab [43].

Recently, modified versions of the monoclonal anti-IgE antibody MEDI4212 with improved binding to FcyRIIIA have been engineered. While the reactivity against IgE remained unchanged the elimination of IgE expressing B-cells in vitro was significantly increased [44]. The afucosylated variant of MEDI4212 decreased serum IgE levels in a humanized mouse model to a higher degree than the fucosylated variant [44]. No clinical human data is currently available for the afucosylated variant of MEDI4212.

Disruptive IgE inhibitors – a new class of anti-IgE molecules

In 2012, a new and promising class of anti-IgE molecules with the ability to not only neutralize free IgE but in addition actively dissociate pre-formed IgE:FcεRI complexes has been reported [45,46]. The disruptive IgE inhibitor, termed DARPin® E2_79, and its improved bivalent version, DARPin® bi53_79, have been demonstrated to desensitize allergic effector cells by actively removing IgE from their cell surface [47]. This mechanism - termed facilitated dissociation - differs from the classic competitive and the allosteric inhibition model [48]. It represents a competitor-induced dissociation mechanism in which the binding site on a ligand becomes exposed during partial ligand:receptor complex dissociation [46]. Interestingly, it has been shown in these studies that Omalizumab also accelerates the dissociation of IgE:FcεRI - however, only at very high concentrations [47]. Recently, the disruptive activity of Omalizumab has been enhanced by introducing three point-mutation into the variable light and constant domain of its Fab fragment, called FabXol3 [34]. The binding sites of FabXol3 and DARPin® E2_79 on the Cε3 domain of IgE are overlapping and of similar size **(Figure 1I, J)**. While E2_79 is acting through facilitated dissociation [46], an allosteric mechanism has been proposed for FabXol3 [34].

Further, a llama-derived humanized single-domain antibody, named 026 sdab, has been described to inhibit IgE binding to FcεRI through an allosteric mechanism by

182 trapping IgE in a closed conformation [49]. The crystal structure of 026 sdab with IgE-
183 Fc_{3-4} revealed no overlap with FcεRI binding sites but significant competition with CD23
184 attachment points on IgE. 026 sdab binds IgE with high affinity (K_D~ 1.4 x 10^{-9} M) and
185 has the ability to disrupt pre-formed IgE:FcεRI complexes **(Figure 1k)**. In line with the
186 observed removal of surface IgE, 026 sdab decreased basophil allergen-sensitivity.
187 Furthermore, 026 sdab has been shown to inhibit binding of IgE:allergen complexes to
188 CD23.
189 Similar to the disruptive IgE inhibitor DARPin® E2_79, the bivalent anti-IgE/ anti-HSA
190 Nanobody® ALX-0962 has been reported to neutralize free IgE and remove FcεRI-
191 bound IgE from human primary basophils [50]. In various studies, it has been
192 speculated that disruptive inhibitors might show faster onset of action compared to
193 conventional anti-IgE molecules and thereby accelerate treatment benefits.
194
195 **Conclusions**
196 In summary, we have highlighted various anti-IgE approaches to interfere with the
197 allergic cascade on multiple levels **(Figure 2a)**. While the neutralization of free serum
198 IgE represents the oldest and most advanced therapeutic strategy, recent studies have
199 paved the way for alternative treatment approaches. Targeting of IgE producing B-cells
200 has gained a lot of momentum. However, it is most likely due to the low frequency, the
201 short half-live and the anatomic location of IgE bearing B-cells that this strategy has
202 shown limited success in clinical trials so far. Disruptive IgE inhibitors that in addition
203 to the neutralization of free IgE actively desensitize antigen-presenting or allergic
204 effector cells are the most recent development in the anti-IgE field. It will be interesting
205 to see whether and how this additional mode of action might translate into patient
206 benefit. The development of a molecule that efficiently targets the allergic cascade at

multiple levels and unifies different modes of action **(Figure 2b)** might be an attractive way to improve the treatment efficacy for allergic disorders in the future.

210 **Table 1.**

	Omalizumab	Ligelizumab	MEDI4212	Quilizumab	bi53_79	026 sdab
Class	IgG1 κ	IgG1 κ	IgG1 λ	IgG1 κ	DARPin*	single-domain antibody
Affinity constant K_D [M]	7 x 10^{-9} M	1.4 x 10^{-10} M	2 x 10^{-12} M	?	12 x 10^{-9} M	1.4 x 10^{-9} M
Free IgE binding	+	++	+++	-	+	+
IgE:FcεRIα complex disruption	+	?	?	-	+++	++
FcεRIα inhibition	+	++	+++	-	+	+
CD23 inhibition	+	?	+	-	+	+
IgE^+ B-cells elimination	-	?	+	+	?	?
References	[25,33,34,47]	[30,36]	[37,38,44]	[40-43]	[33,46,47]	[49]

211
212 * DARPin = Designed Ankyrin Repeat Protein; + = positive evidence; - = negative evidence; ? = unknown

Figure Legends

Figure 1. Structural representations of IgE-Fc variants alone or in complex with the indicated receptors or anti-IgE molecules. The two IgE-Fc heavy chains are represented in yellow and black, while IgE receptors and anti-IgE molecules are colored in red or red/orange. **(a)** Asymmetrically bent conformation of IgE-Fc_{2-4}, PDB-ID: 1O0V; **(b)** Complexed FcεRIα:IgE-Fc_{3-4}, PDB-ID: 1F6A; **(c)** Open conformation of IgE-Fc_{3-4}, PDB-ID: 3HA0; **(d)** Complexed FcεRIα:IgE-Fc_{2-4}, PDB-ID: 2Y7Q; **(e)** Complexed CD23:IgE-Fc_{3-4}, PDB-ID: 4EZM; **(f)** Closed conformation of IgE-Fc_{3-4}, PDB-ID: 3H9Z; **(g)** Complexed Omalizumab Fab:IgE-G335C-Fc_{3-4}, PDB-ID: 5HYS; **(h)** Complexed MEDI4212:IgE-Fc_{3-4}, PDB-ID: 5ANM. Structure is vertically turned 90° to the left compared to all other images; **(i)** Complexed DARPin E2_79:IgE-Fc_{3-4}, PDB-ID: 4GRG; **(j)** Complexed FabXol3:IgE-Fc_{2-4}, PDB-ID: 5G64; **(k)** Complexed 026 sdab:IgE-Fc_{3-4}, PDB-ID: 5NQW.

Figure 2. Anti-IgE intervention strategies. During allergic sensitization, activated isotype switched B-cells (B) produce allergen-specific IgE, which is released into the circulation. Soluble free IgE may bind to the low affinity IgE-receptor CD23 expressed on B-cells or to the high affinity IgE-receptor FcεRIα expressed on allergic effector cells such as basophils (Ba) in the blood. **(a)** The primary mode of action for the anti-IgE antibodies Omalizumab, Ligelizumab and MEDI4212 is the neutralization and clearance of soluble IgE (red solid lines). Omalizumab and MEDI4212 also inhibit binding of IgE to CD23 (dashed lines). Further, Omalizumab accelerates the dissociation of IgE from FcεRIα (dashed line), while the afucosylated version of MEDI4212 aims to target and eliminate IgE^+ B-cells. Quilizumab targets the membrane proximal M1 domain on IgE^+ B-cells and eliminates these cells by ADCC; black star:

239 improved FcγRIIA binding. **(b)** We propose, that an anti-IgE molecule which interferes
240 with the allergic cascade at multiple levels would achieve maximal therapeutic efficacy.
241 Ideally, such a molecule would neutralize soluble IgE, actively dissociate pre-formed
242 IgE:FcεRIα complexes on the surface of sensitized allergic effector cells and target
243 IgE-producing B-cells to inhibit IgE-synthesis.
244

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Figure 1.

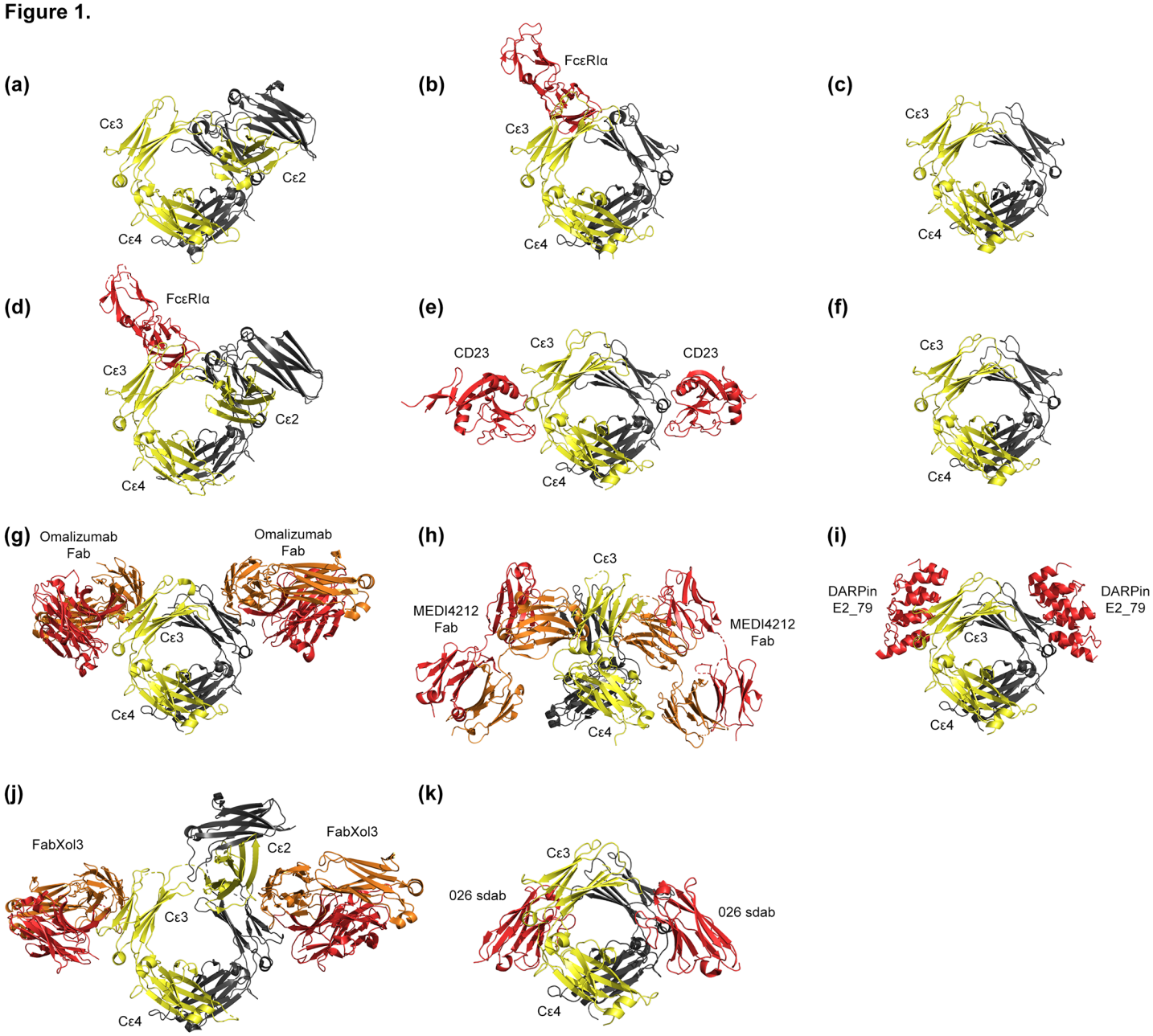

Figure 2.