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The role of IgE, IgG, and IgA in tolerance, sensitization, and targeted treatment of allergic disease

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Abstract

Immunoglobulin E (IgE)-mediated allergy is the most common hypersensitivity disease affecting more than 30% of the population. In genetically-predisposed subjects exposure to minute quantities of allergens leads to the production of IgE antibodies which is termed allergic sensitization and mainly occurs in early childhood. Allergen-specific IgE then binds to the high (Fc?RI) and low affinity receptors (Fc?RII, also called CD23) for IgE on effector cells and antigen-presenting cells, respectively. Subsequent and repeated allergen exposure increases allergen-specific IgE levels and, by receptor cross-linking, triggers immediate release of inflammatory mediators from mast cells and basophils whereas IgE-facilitated allergen presentation perpetuates T cell-mediated allergic inflammation. Due to engagement of receptors which are highly selective for IgE even tiny amounts of allergens can induce massive inflammation. Naturally occurring allergen-specific IgG and IgA antibodies usually recognize different epitopes on allergens compared to IgE, and do not efficiently interfere with allergen-induced inflammation. However IgG and IgA antibodies to these important IgE epitopes can be induced by allergen-specific immunotherapy or by passive immunization. These will lead to competition with IgE for binding with the allergen and prevent allergic responses. Similarly, anti-IgE treatment does the same by preventing IgE from binding to its receptor on mastcells and basophils. Here we review the complex interplay of allergen-specific IgE, IgG and IgA and the corresponding cell receptors in allergic diseases and its relevance for diagnosis, treatment and prevention of allergy.

Invited review: The role of IgE, IgG, and IgA in tolerance, sensitization, and targeted treatment of allergic disease

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1. Abstract:

Immunoglobulin E (IgE)-mediated allergy is the most common hypersensitivity disease affecting more than 30% of the population. In genetically-predisposed subjects exposure to minute quantities of allergens leads to the production of IgE antibodies which is termed allergic sensitization and mainly occurs in early childhood. Allergen-specific IgE then binds to the high (FcεRI) and low affinity receptors (FcεRII, also called CD23) for IgE on effector cells and antigen-presenting cells, respectively. Subsequent and repeated allergen exposure increases allergen-specific IgE levels and, by receptor cross-linking, triggers immediate release of inflammatory mediators from mast cells and basophils whereas IgE-facilitated allergen presentation perpetuates T cell-mediated allergic inflammation. Due to engagement of receptors which are highly selective for IgE even tiny amounts of allergens can induce massive inflammation. Naturally occurring allergen-specific IgG and IgA antibodies usually recognize different epitopes on allergens compared to IgE, and do not efficiently interfere with allergen-induced inflammation. However IgG and IgA antibodies to these important IgE epitopes can be induced by allergen-specific immunotherapy or by passive immunization. These will lead to competition with IgE for binding with the allergen and prevent allergic responses. Similarly, anti-IgE treatment does the same by preventing IgE from binding to its receptor on mastcells and basophils. Here we review the complex interplay of allergen-specific IgE, IgG and IgA and the corresponding cell receptors in allergic diseases and its relevance for diagnosis, treatment and prevention of allergy.

2. Introduction:

The discovery of immunoglobulin (Ig) E in the mid-1960' by two independent groups led by Kimishige Ishizaka in the United States of America and S.G.O Johansson in Sweden resulted in a significant impact on the diagnosis and management of allergic diseases[1,2]. Since then, IgE has been shown to play an essential role in type I immediate allergic responses[3,4]. Antibody isotype class switching in favour of IgE can occur locally in the nasal mucosa[5]and bronchial tissues[4,6,7]. Dendritic cells (DCs) present in the upper layers of the epithelium and lamina propria of the airways[8,9], gut and the skin are well disposed to capture allergens and drive T cell polarisation towards a pro-allergic-type II immune response. DCs migrate to the draining lymph nodes, where they prime and activate naïve T cells to differentiate, proliferate and

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clonally expand into Th2, and follicular T helper cells that produce interleukin-4 (IL-4) and interleukin-13 (IL-13) and IL-21, which lead to the differentiation and clonal expansion of naïve T cells to Th2 cells. However, earlier studies demonstrating that also B cells may be important APCs in the initiation of IgE sensitisation[10,11] are supported by more recently published studies[12,13]. Moreover, the enhanced expression of Th2 cytokines such as IL-4 and IL-13 produced by mast cells and basophils [6,7,14] in the nasal mucosa can promote tissue mast cells to induce IgE synthesis in B cells in an indirect manner, resulting in local IgE synthesis by B cells[5,15]. In turn, after sensitization, IgE can also enhance Th2 cell response in a FcεRI and CD23-dependent manner[16-19]. It is noteworthy that there is evidence that (non-IgE) allergen-specific antibodies in early life can modulate allergic sensitisation. During pregnancy and through breastmilk, maternal immunoglobulins are transferred to offspring and it seems that maternal allergen-specific IgG may protect the off-spring from allergic sensitization[20-22]. Birth cohorts and studies in animal models have revealed a long-term influence on offspring allergy susceptibility[21,23]. Restoration of immune tolerance following long-term allergen immunotherapy is associated with the induction of local and systemic IgG and IgA associated neutralising antibodies[24-27]

This article reviews the role of IgE, IgG and IgA in allergic inflammation and induction of immune tolerance in early life as well as after allergen immunotherapy. Furthermore, targeting of IgE with anti-IgE antibodies as well as the effects of passive immunization with allergen-specific IgG is considered and discussed.

3. IgE and its receptors

3.1 Immunoglobulin E (IgE)

Structurally, in agreement with other antibody classes, IgE antibody comprises two identical light and heavy chains. Each chain is formed of 110 amino acid "immunoglobulin domains". Disulfide bonds covalently link the light and heavy chains. Unlike IgD, IgG and IgA, which have three constant region domains, the heavy chain is structurally similar to the μ heavy chain of IgM as it has four constant region domains (Cε1-Cε4, see Figure 1). Cε3 and Cε4 domains are homologous in both sequence and quaternary structure to the Cγ2 and Cγ3 domains of IgG antibody isotype[3]. IgE can be distinguished from IgG by the position of its Cε2 domains substituting the hinge region of IgG. The hinge region of IgE is susceptible to digestion by papain. The two antigen-binding sites are formed by pairing of the variable region of light and heavy chains.

IgE is asymmetrically bent at the Cε2-3 linker and folds on itself with the two Cε2 domains folded back and almost touching the Cε4 domains[28-30] (Figure 1.2). Fluorescence resonance energy transfer (FRET) analysis has revealed that the distance between N- and C- terminal of IgE is around 10 Å, and that binding of IgE with its receptor induces conformational changes that increase this distance considerably.

Immunoglobulin E is central to type I immediate allergic responses[1,3]. Several studies have illustrated that antibody isotype class switching in favour of IgE may occur locally in the nasal mucosa in allergic rhinitis patients and in lymphatic tissues adjacent to sites of allergen contact but the precise sites for IgE production are not yet known[5-7,31,32]. Elevated concentrations of IgE antibodies have been demonstrated in target organs, reaching over ten times more in atopic and allergic individuals than non-atopics[5,7,33,34]. IgE antibodies bind with high affinity to FcεRI (association constant, Ka = 10^{10} M-1) on mast cells and basophils' surface.

3.2 ΦςϵPI – στρυςτυρϵ ανδ φυνςτιον ον ϵφφϵςτορ ςϵλλς ανδ ΑΠϚ

The high-affinity IgE receptor (FcεRI) is a member of the immunoglobulin (Ig) superfamily. It is highly expressed as an αβγ2 tetramer (˜200,000 molecules/cell) on the surface of mast cells and basophils[35,36]

It consists of four polypeptide chains, an α chain, a β chain and two disulfide-linked γ chains[37](Saini *et al.,*2001). In human monocytes, Langerhans" cells and peripheral blood DCs, eosinophils, platelets and smooth-muscle cells, FcεRI is expressed as a αγ2 trimer, consisting of one α and two γ chains[3,38]. The α chain consists of an extracellular domain, a single transmembrane helix domain and a short cytoplasmic sequence. The IgE binding function of the high-affinity IgE receptor is confined to the two extracellular domains of the α chain, with a 1:1 binding stoichiometry. Both intracellular sequences of the β and γ chains consist of immunoreceptor tyrosine-based activation motifs (ITAMs). The β subunit chain functions as an

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amplifier of downstream events after the initial activation of surface FcεRI[37] (Saini *et al.,* 2001). The lack of a β chain may account for the variable expression of this receptor on certain cells. Cross-linking of tetrameric FcεRI on the surface of mast cells and basophils leads to cellular activation, resulting in degranulation and the release of preformed mediators, synthesis of lipid mediators and the release of inflammatory cytokines, leading to the recruitment of leukocytes which further enhance the allergic response[39]. IgE enhances the expression of FcεRI by stabilization of the receptor[40,41], and occupancy of FcεRI can also prolong mast cell survival by IgE[42]

The trimeric expression of FcεRI on monocytes, DCs and Langerhans cells has been shown to facilitate allergen presentation to CD4+ T cells. The efficiency of FcεRI-mediated allergen uptake by antigen-presenting cells (APCs) is 100 to 1000-fold more effective than any endocytosis or pinocytosis[17,43]. FcεRI is up-regulated by mast cells in seasonal allergic rhinitis and its expression correlates with serum IgE concentrations[44-47].

3.3 CD23 - structure and function on B cells

The low-affinity IgE-receptor FcεRII, also known as CD23, is a single chain type II integral membrane protein of 45kD and belongs to the C-type (Calcium-dependent) lectin superfamily [3,48-50]. The membrane-bound CD23 consists of three lectin "head" domains spaced from the membrane by a three ∝-helical coiled-coil "stalk". The lectin head domains of CD23 in the human form contain the C-terminal tail sequence. The stalk region is susceptible to proteolytic cleavage. Adam 10, a desintegrin and a metalloproteinase has been shown to release soluble CD23 (sCD23). This proteolytic cleavage results in trimeric fragments of CD23 (37kD) containing the stalk or monomeric fragments (25kD, 16kD) lacking the stalk[51]. CD23 recognises the protein rather than the carbohydrate moiety of IgE. A single lectin head fragment can bind to the IgE-Fc portion with an affinity of ka $=10^6$-10^7 M-1 while the avidity of the trimeric CD23 to bind IgE-Fc results in the overall high-affinity binding (Ka = 10^8 -10^9 M-1)[51,52]. Two isoforms of CD23 which differ by seven (CD23a) or six amino acids (CD23b) have been defined. CD23a is constitutively expressed on antigen-activated B cells, and IL-4 induces CD23b expression in several cell types, including B cells and epithelial cells[3,53,54].

4. Physiological role of IgE in allergic inflammation

4.1 Role of IgE on mast and basophils responses.

Mast cells and basophils were identified in tissue and blood, respectively, by Ehrlich almost 200 years ago, and their function was anticipated during this period[55] describes early findings by Ehrlich). However, the functional relationship between these cells was not described in detail until after the discovery of IgE. Follow-up experiments by T. and K. Ishizaka revealed that both cells were activated through the high-affinity IgE receptor in the presence of IgE. Allergen-induced cross-linking of IgE bound to FcεRIs on the surface of mast cells or basophils induce aggregation of the receptor and intracellular signalling events[56] that leads to Ca+-dependent release of preformed mediators and *de novo*synthesis and secretion of lipid mediators and cytokines (e.g. IL-4 and IL-13)[57,58] (Figure 2). The concentration of serum IgE regulates the FcεRI surface expression on these effector cells, and this feedback mechanism can reduce the allergen concentration needed for activation[59]. Moreover, recent findings suggest that IgE's glycosylation (sialylation) may be critical for the activation of mast cells in a mouse model of anaphylaxis based on IgE[60]. The essential role of IgE cross-linking that leads to activation of effector cells has obtained less attention but was described in detail for basophils by Christensen et al.[61] and later similar observations were made for mast cells[62]. These studies confirmed that the concentration of allergen-specific IgE, IgE affinity and the ratio of allergen-specific to total IgE are the governing factors for the strength of effector cell release. The effect of specific to total IgE ratio and the observation that one high-affinity IgE in a mixture overrides the difference between high, medium, and low-affinity IgE may explain why correlations between concentrations of allergen-specific IgE and clinical symptoms has been difficult to establish[63]. Detailed studies with model antigens demonstrate that the spacing between the epitopes is critical[64] and the number as well as relative positioning of IgE epitopes on allergens[65,66] may also be critical for the ability to cross-link the receptors, which adds to the complexity of IgE-mediated effector cell activation.

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4.2 Role of IgE in enhancing T cell responses.

The concept of IgE-facilitated allergen presentation was first elucidated in studies that showed that complexes of specific IgE with allergens could significantly enhance the responses of allergen-specific T cells at low allergen concentrations[16,19,67]. This IgE mediated allergen presentation, or facilitated allergen presentation, involved binding the IgE-allergen complexes to CD23 on antigen-presenting B cells (Figure 2).

Around the same time, it became apparent that dendritic cells and monocytes from peripheral blood express the high-affinity IgE receptor (FcεRI) and could also activate allergen-specific T cells in an IgE-facilitated manner[17,52]

These findings are relevant because allergen levels in the respiratory tract are extremely low upon natural allergen exposure. The IgE-facilitated presentation of allergens to T cells enables T cell activation at these low allergen exposures.

The binding of allergen-IgE complexes to antigen-presenting cells is dependent on several parameters. In principle and in a model system, monoclonal IgE is sufficient to present allergen[68]. Furthermore, the complexity of IgE binding to multiple epitopes on allergens and their affinity has been shown to correlate with the facilitation of T cell responses[69]. These findings suggest that the number of IgE molecules bound per allergen may play an essential role in this complex formation and binding. This was confirmed recently in a study by Villazala-Merino et al.[70] where non-FcεRI cross-linking monoclonal IgE-monomeric allergen complexes, i.e. (one IgE molecule binding two Bet v1 molecules) could enhance T cell activation. However, this activation was further enhanced by 100-fold when cross-linking IgE-allergen oligomer complexes were used (multimeric complexes). Finally, the heterogeneity of allergen epitopes recognized by IgE, the presence of competing IgG(4) antibodies, the density of CD23 on the surface of B cells in peripheral blood of allergic patients correlates with the ability to enhance T cell activation by allergen-IgE complexes [71].

5. The role of IgG and IgA in tolerization and treatment of IgE-mediated allergies

5.1 The role of IgG and IgA in preventing sensitisation in early life

Maternal IgA and IgG antibodies from breast milk or transferred over the placenta during pregnancy, play an important role in the development of allergy in the offspring (summarized in Figure 3). During the third trimester of pregnancy, IgG immunoglobulins are transferred from the placenta into the serum of the fetus using the non-classical neonatal Fc Receptor (FcRN). These IgG antibodies are thought to be important for providing protection to infants from infectious disease[22,72]. Maternal IgG to airborne allergens (i.e. House dust mite, Birch pollen, cat) and food allergens (egg, cow milk) were also found to be transferred in utero in birth cohorts[73,74]. High levels of cord blood IgG antibodies to cat and birch, but not to food allergens, were associated with less atopic symptoms in the children during the first eight years of life[21,73] Maternal allergen immunotherapy has also resulted in the induction of allergen-specific IgG in the serum of the offspring, further confirming they are passively transferred across the placenta into the fetus[75,76]. However, a review of five studies of allergen-specific immunotherapy during pregnancy did not show any clear evidence of allergy reduction in the offspring[77] .

In addition to IgG, also maternal IgE can be transported over the placenta via FcRN, resulting in IgE binding to already competent mast cells in the fetus.[78]

Several studies have reported that following birth, mothers continue to transfer IgG in addition to secretory IgA to their offspring through breast milk[22,79]. Antibodies to both airborne and food allergens have been detected in human milk[74,80,81]. Maternal allergen-specific IgG can be detected in children's serum up to 6 months of age, and the specificity to the allergen in plasma, breast milk and cord blood is quite similar[21]. It is noteworthy that infants of mothers with high concentrations of allergen-specific IgG in serum and breastmilk did not show sensitisation to the allergen at five years. More importantly, sensitised children had mothers with low concentrations of allergen-specific IgG[21].

For four decades, rodent experiments have explored the impact of in utero and of milk transfer of IgG

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to offspring on allergy sensitisation and their mechanisms of action[82]. Neutralisation of the allergen and allergen-specific modulation of B and T cell regulatory properties of maternal IgG antibodies have been described[82]. In addition to possible immune regulation induced by the sole presence of maternal IgG, maternally derived immune complexes made of allergen bound to IgG may also be critical for regulation of long-term allergy susceptibility. Allergen-IgG immune complexes have been detected in cord blood[83,84] and human milk[81,84]. There is strong evidence from rodent experiments that allergen-IgG immune complexes in breast milk are very potent in eliciting an immune response in offspring. Oral exposure to OVA-IgG immune complexes through breast milk resulted in the induction of OVA-specific Forkhead box protein P3 (FOXP3) regulatory T cells (Tregs) responsible for prolonged tolerance to OVA in offspring subsequently leading to respiratory and food allergy prevention[20,81] . This appeared to result from a protected transport of OVA across the gut barrier and an enhanced presentation by dendritic cells, both depending on the use of the neonatal Fc Receptor (FcRn).

A recent report analysed the influence of maternal immune status on the induction of protection against cow milk allergic sensitisation upon β-lactoglobulin (β-LG) transfer through breast milk. Using two different protocols for maternal immunisation, the study showed that the levels of antibodies in breast milk positively correlated with the inhibition of allergic sensitisation in offspring and no protection was induced by the antigen transfer only[85]. Similarly, maternal exposure to peanut during breastfeeding inhibited allergic response to peanut in offspring only when mothers had been immunised but not if naïve to peanut[86,87]. However, allergen transfer to offspring in the presence of maternal antibodies does not systematically result in tolerance induction, as shown for House dust mite (HDM) allergen[88]. A study in mice showed that mice nursed by HDM-exposed mothers exposed developed a gut immunity imbalance associated with the expansion of Th2 cells and a refractory state to oral tolerance. Importantly, when neutralising HDM protease activity, this deleterious effect on gut immune ontogeny in offspring was abolished[89]. This observation highlights the importance of the biological properties of the allergen itself, as in the case of HDM, the proteolytic activity of the allergens was responsible for immune priming[89].

In addition to human breast milk, allergen-specific IgG (bIgG) has been detected in cow's milk[90]. It is not clear if allergen-specific IgG is complexed to allergens in the milk. However, after oral ingestion, the IgG can theoretically bind to allergens that are swallowed, and thereby play a role in tolerisation to the allergen, as has also been noted in epidemiological studies on the consumption of raw milk and allergies[91-93].

In addition to allergen-specific IgG, there is some evidence that allergen-specific IgA in breast milk is associated with protection as shown for infants' cow's milk allergy[94-97]. The total levels of IgA in breast milk are inversely associated with AD in early life[98]. A recent a study reported that in mice maternal milk IgA might play an important role in establishing a gut regulatory T cell setpoint in offspring gut and thereby tuning gut immune responses and inflammatory disease susceptibility[99].

5.2 Induction and function of allergen-specific IgG and IgA by allergen immunotherapy

Allergen immunotherapy involves the repeated administration of allergens or allergen products to IgE-sensitised allergic individuals to induce long-term tolerance on subsequent exposure to the offending allergen(s)[100]It is indicated in patients with symptoms on exposure to relevant allergens and failure to respond to regular use of anti-allergic drugs. AIT has been shown to be effective for allergic rhinoconjunctivitis, allergic asthma and anaphylaxis due to venom of stinging insects. AIT traditionally involves subcutaneous injections of allergen extracts weekly then monthly for 3 years. Daily administration via the sublingual route has been shown to be an effective and safer alternative [101]. Strategies to improve efficacy, reduce side effects and enable shorter more convenient immunotherapy protocols are desirable[102]. These have included alternative routes (eg epicutaneous, intralymphatic) use of short T cell peptides, medium chain length hydrolysed or synthetic peptides, combination products of allergen with Toll-like receptor agonists or biologics and recombinant major allergen mixtures or hypoallergenic variants. So far these strategies have failed to deliver outcomes over and above currently available products[103].

Allergen immunotherapy has been shown to be accompanied by increases in allergen-specific antibodies.

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Cooke originally identified passive transfer of a serum factor that provided protective immunity to ragweed following successful ragweed immunotherapy[25] This was subsequently shown to reside within the immunoglobulin IgG fraction, long before IgE was discovered.

For pollen AIT, an initial transient rise in specific IgE is followed by blunting of seasonal IgE increases and a gradual decline over several years[104]. Both SCIT and SLIT result in increases in allergen-specific IgG1/IgG4, and specific IgA1/IgA2[105]. These antibodies increase at 2-6 months and are detectable both in blood and in local target organ secretions, for example in nasal fluid [106]. Whereas SCIT induces largely IgG responses, a recent head-to-head trial showed that SLIT induces preferential allergen-specific[27] IgA1/2.

A major advance has been the availability of recombinant major and minor allergenic components that enable an accurate molecular diagnosis. There is a strong case that measurements of allergen-specific antibodies to standardised whole extracts could be supplemented by molecular diagnosis using individual allergen molecules to discriminate between antibodies binding to allergens and non-allergenic extract components[107,108]. Whether standardised allergen extracts will be replaced or supplemented by tailor-made recombinant mixtures/ hypoallergenic variants based on individual molecular profiles remains to be tested.

IgG4 and other human IgG subclasses are similar in structure but have differences in binding to accessory molecules and receptors, altering their functionality. IgG4, in particular, induced following chronic antigen responses co-exist as two isomers diverging in their disulfide bonds of hinge cysteines. There is clear evidence that *in vivo* , half-molecules of IgG4 can recombine randomly with other half-molecules of IgG4, resulting in monovalent-bispecific antibodies[109,110]. As a consequence, IgG4 is unable to efficiently cross-link target allergen and form immune complexes. It is unable to bind with both Fab arms to a multivalent antigen, leading to a lower avidity. IgG4 has low affinity for activating FcγR whilst retaining high affinity for the FcγRIIb. These characteristics enable IgG4 to be an efficient inhibitor of IgE-dependent reactions without untoward inflammation associated with igG immune complex formation and complement activation.

Allergen-specific IgA2 and polymeric IgA2 has also been shown to be elevated following grass pollen SCIT. Polymeric IgA2 was purified from post-immunotherapy serum and used to passively sensitize autologous monocytes. Subsequent cross-linking *in vitro* of IgA on monocytes by antigen or anti-IgA resulted in IL-10 production, supporting an alternative role for IgA antibodies in inducing tolerance following AIT[111].

Immunoreactive IgG and IgA antibodies are elevated after AIT but have correlated poorly with the clinical response to treatment. This may be explained in part by responses to non-allergenic proteins or to irrelevant minor or cross-reactive allergens and this can be addressed by measuring major allergen components[105,112]. However, at least as relevant, immunoreactive antibodies relate largely to allergen exposure during AIT and may have no bearing on the affinity and/or avidity of these antibodies in blocking the formation of allergen-IgE complexes and hence blocking IgE responses. This highlights the importance of using functional antibody assays to supplement immunoreactive IgG and IgA assays.

Allergen-specific IgG4 (and likely other antibody isotypes) compete with IgE for allergen and prevents the formation of allergen-IgE complexes from binding to FcεRI on effector cells (mast cells, basophils and dendritic cells) and to FcεRII (CD23) on B cells (Figure 2). van Neerven originally demonstrated that serum obtained after birch pollen immunotherapy inhibited IgE-facilitated allergen presentation by B cells to an allergen-specific T cell clone, with decreased specific clonal T cell proliferation and cytokine production[113]. This was confirmed by further studies of birch immunotherapy[114,115]. confirmed increases in serum IgG-associated blocking activity for IgE-FAB in grass pollen immunotherapy[116]. That persisted for years after discontinuation along with clinical benefit (and by affinity chromatography showed that the inhibitory factor resided largely but not exclusively within the IgG4 fraction[117]. Recent data supports a putative role for allergen-specific IgG2[118]as a blocking factor for IgE-mediated reactions. Shamji validated the IgE-FAB assay and showed that serum IgE-FAB increased in a time- and dose-dependent fashion after grass pollen AIT[119,120]and correlated more closely with clinical response than accompanying elevated IgG4 levels This raised the possibility for IgE-FAB inhibition to predict individual responses to AIT[105]. Such blocking antibodies could also prevent captured allergen from stimulating IgE-producing cells thereby reducing boosts

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of IgE production caused by allergen exposure[70,121,122].

The functional role of serum blocking antibodies after AIT has also been illustrated by inhibition of IgE-mediated basophil activation (Figure 2). After grass pollen AIT, post-immunotherapy serum inhibited basophil histamine release with a time-course that paralleled inhibition of IgE-FAB and correlated with inhibition of the immediate skin response to grass pollen at 8-16 weeks[123]. This was also shown using Bet v 1-specific IgG1 and IgG4 antibodies after birch pollen AIT[124] In a murine model this inhibitory effect of IgG was mediated via the FcγRIIB receptor. However, an[125]tibodies directed against FcγRII did not prevent serum IgG-mediated inhibition of basophil activation following birch AIT, implying that direct competition with IgE for allergen rather than activation of FcγRII-mediated inhibition of downstream IgE receptor signalling pathways was responsible[125].

During grass pollen AIT inhibition of basophil activation has been shown by suppression of surface CD63 expression and by increases in intracellular Diamine Oxidase as detected by whole blood flow cytometry[126]. Suppression of basophil activation has also been shown for birch pollen immunotherapy (REF), as well as following venom immunotherapy[127].

The therapeutic potential of blocking antibodies following AIT is highlighted by a recent study of passive immunotherapy in cat allergic individuals who received a single dose of two synthetic anti-Fel d 1 specific IgG4 antibodies that resulted in inhibition of the nasal response to a standardized cat whole allergen extract that persisted for twelve weeks[128,129].

6. IgE and IgE-receptor targeting therapies for treating allergies

Another group of antibodies that prevent histamine release by basophils and mast cells are the anti-IgE antibodies. They exert their effect by preventing IgE from binding to FcεRI and CD23 (Figure 1 and 2). Binding of IgE to CD23 may involve different portions of CD23 and, interestingly can be blocked with Omailizumab which also blocks IgE binding to the high affinity receptor for IgE [71]. In addition, anti-IgE has a similar inhibitory effect as AIT-induced IgG and IgA antibodies that block IgE-mediated T cell activation[130].

The structures of the ectodomain regions of FcεRI and CD23 in complexes with IgE-Fc have revealed how these two distinct receptors interact with IgE[97,131,132]. IgE binding to its two receptors is regulated through unique conformational changes in the IgE-Fc domain that enable an allosteric competition between low and high-affinity receptors[131,133]. IgE binding to FcεRI occurs through the tips of the two IgE Cε3 domains, engaging both antibody heavy chains in an asymmetric "open" conformation [132,133] In contrast, CD23 binding occurs to a distinct surface of the IgE-Fc at the junction between Cε3-Cε4 domains and favours a "closed" conformation that inhibits FcεRI binding[131]. High affinity binding to FcεRI leads to the prebinding of serum IgE to receptor-expressing cells, sensitizing them to respond upon allergen exposure and cross-linking. In contrast, IgE binding to CD23 is of lower affinity and is stabilised through avidity effects, most notably by IgE-allergen complex formation. Strikingly, IgE bound to FcεRI is incredibly stable, persisting on peripheral mast cells for weeks-months and impacting the safety and speed of AIT/OIT approaches.

Two anti-IgE antibodies, omalizumab and ligelizumab[134,135] have been advanced as therapeutics for the treatment of allergic diseases, including allergic asthma, chronic spontaneous urticaria, chronic rhinosinusitis and food allergies. However, other anti-IgE antibodies are in clinical development (e.g. Xmab7195/UB221/omalizumab biosimilars). Omalizumab and ligelizumab highlight the impressive impact that anti-IgE can have in allergy treatment[136]. Omalizumab was the first anti-IgE developed as a therapeutic, initially for the treatment of severe allergic asthma in 2003. Since then, omalizumab has shown efficacy in treating CSU, food allergy and chronic rhinosinusitis[137]. As discussed elsewhere in this review, omalizumab enhanced OIT treatment in food allergy clinical trials, reducing allergen challenge reactions and enabling a more rapid increase in allergen dosing and simultaneous tolerization for multiple allergens[138]. Ligelizumab is a next-generation, higher affinity anti-IgE that shows an improved ability to suppress free IgE in patients[135]. Despite having an ˜100-fold higher affinity for IgE, ligelizumab surprisingly did not show improved efficacy in treating allergic asthma patients[139,140]. However, in phase II clinical studies, ligelizumab showed improved

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efficacy over omalizumab for the treatment of CIU[141]. It remains to be established whether ligelizumab will have a significant benefit in OIT/AIT relative to omalizumab.

The structures and mechanisms of omalizumab vs ligelizumab are revealing and provide insight into the possible differences in their therapeutic impact. Omalizumab and ligelizumab both engage epitopes in the IgE Cε3 domains adjacent to the binding site for FcεRI[139,142,143]. Despite the substantial overlap in their epitopes, ligelizumab binds across the IgE dimer engaging residues in both Cε3 domains and overlapping the space that would be occupied by FcεRI. In contrast, omalizumab engages an epitope towards an outer face of the Cε3 domains, does not bind across the IgE dimer and lies somewhat peripherally to FcεRI. One of the consequences of these distinct binding interactions is that omalizumab can effectively inhibit binding to FcεRI and CD23, while ligelizumab shows preferential inhibition of FcεRI[139]. The ability of ligelizumab to block CD23 binding is weaker than omalizumab, despite its much higher IgE affinity. The weaker inhibition of IgE:CD23 interactions exhibited by ligelizumab may account for its failure to outperform omalizumab in clinical trials for allergic asthma [139,140], where CD23 is thought to play an essential role in disease through antigen presentation and or antigen transport[144,145]. CD23 has also been studied as a target in allergic diseases. However, although a phase 1/2 study with the anti-CD23 mAb Lumiliximab in asthma patients showed a good safety profile, anti-CD23 has not been developed further in asthma or allergy [146].

It will be exciting and informative to compare the activities of omalizumab and ligelizumab in AIT, which may help assess the clinical importance of the inhibition of CD23 and FcεRI interactions during tolerization to food or other allergens.

The rationale of combining anti-IgE with AIT or OIT is that the combination may prevent allergic side effects of AIT[130] and OIT, allow more rapid updosing of allergen, and will provide immediate clinical benefit. Since 2007, several studies have addressed this combination treatment. These are reviewed in detail in [138,148]. Overall, both these combination treatments have shown promising results, especially evidenced by decreased adverse reactions to AIT and OIT. Larger follow up studies are needed to define the optimal dosing and target groups for this type of combination treatment.

Finally, a class of "disruptive" IgE inhibitors has been described based on Designed Ankyrin Repeat Proteins (DARPins), which can rapidly dissociate FcεRI-bound IgE in vitro and in vivo[149,150]. Such kinetically active anti-IgE inhibitors may have the potential to rapidly desensitise peripheral mast cells and significantly accelerate the timelines for AIT in the future.

7. Future perspectives

The important role of IgE in type 1 allergic diseases has been known for a very long time. The functional role of allergen-specific IgG and IgA antibodies induced by AIT has shown their ability to interfere with the interaction of IgE with the allergen. In addition, transplacental or breastfeeding-mediated transfer of immune complexes of maternal IgG with allergens to the fetus may protect against sensitization to allergens in early life.

The knowledge we have gained over the last two decades has been instrumental in developing novel therapeutic approaches by targeting IgE itself with anti-IgE antibodies or receptor-targeting antibodies, enhancing blocking antibodies by AIT or even passive transfer of allergen-specific IgG to allergic patients (see Box 1 for methods used to measure these allergen-specific antibodies and their function in more detail). This knowledge may help to further establish the relevance of blocking antibodies as a biomarker for clinical effects of AIT.

Finally, this may lead to future therapeutic approaches such as combination treatments with therapeutic antibodies and AIT or OIT (e.g. combination with anti-IgE, allergen-specific IgG, or cytokine-directed antibody therapies), as well as preventive approaches such as maternal allergen vaccination to enhance delivery of allergen-specific IgG and IgA antibodies during pregnancy and early life to prevent sensitization to respiratory and food allergens.

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9. Legends to figures

Figure 1. IgE and its receptors.

IgE antibody uses two identical light and heavy chains and the constant region has four domains (Cε1-Cε4). The two antigen-binding sites are formed by pairing of the variable region of light and heavy chains. IgE is asymmetrically bent at the Cε2-3 linker and folds on itself with the two Cε2 domains folded back and almost touching the Cε4 domains. IgE interacts with the high affinity IgE receptor FcεRI with the Cε2 and Cε3 domains, and with the low affinity IgE receptor CD23 with the Cε3 and Cε4 domains. Anti-IgE antibodies like Omalizumab binds to the Cε3 domain of IgE and can therefore inhibit the binding of IgE to both FcεRI as well as to CD23.

Figure 2. IgE-mediated Th2 and Mast cell/basophil activation and inhibitory effects of allergen-specific IgG and -IgA as well as anti-IgE.

Inhibition of IgE-mediated Th2-cell activation (left panel) and basophil/mast cell degranulation (right panel) by allergen-specific IgG and -IgA (purple), and anti-IgE (red) treatment. Whereas allergen-specific IgG and IgA compete with IgE for binding to allergens, anti-IgE antibodies bind to IgE and block binding of IgE to both the high affinity (FcεRI) and low affinity (CD23) receptors for IgE expressed on antigen presenting cells and basophils/mast cells. In this way they can inhibit IgE-mediated activation of allergen-specific T cells as well as the release of inflammatory mediators by basophils/mast cells induced by IgE-mediated crosslinking of FcεRI after allergen exposure

Figure 3. Maternal immunoglobulin-mediated imprinting of allergic responses in the offspring.

Maternal IgG (blue) to airborne allergens and food allergens reach the offspring in utero by a transfer across the placenta and after birth through breast milk and transfer across the gut. The neonatal Fc receptor (FcRn) carries maternal IgG either free or bound to allergen. Free IgG can inhibit allergic sensitisation in offspring by modulating B cell reactivity. Allergen-IgG immune complexes can induce immune tolerance by promoting allergen specific Treg expansion . Maternal IgE (purple) might also be transported across the placenta by FcRn. Fetal mast cells bear the IgE receptor (FcεR1) and bind maternal IgE. In mice, these IgE-loaded fetal mast cells are functionally competent, degranulate upon exposure to allergen, and persist in neonates, in whom they may mediate allergic disease in early life. Maternal secretary IgA (orange) are also found in human breast milk and might decrease allergic sensitisation by controlling allergen transfer across offspring gut. Evidence in mice also suggest they might control the expansion of Tregs in offspring.

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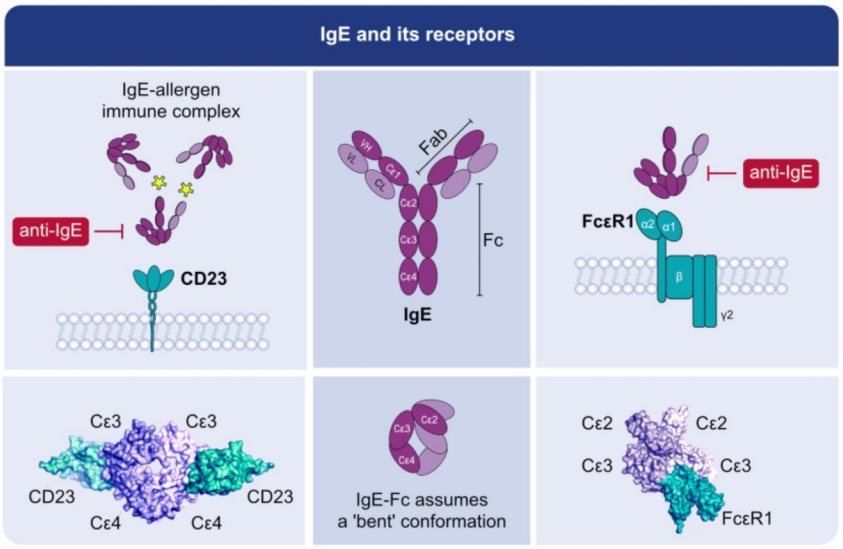

Figure 1 Shamji et al.

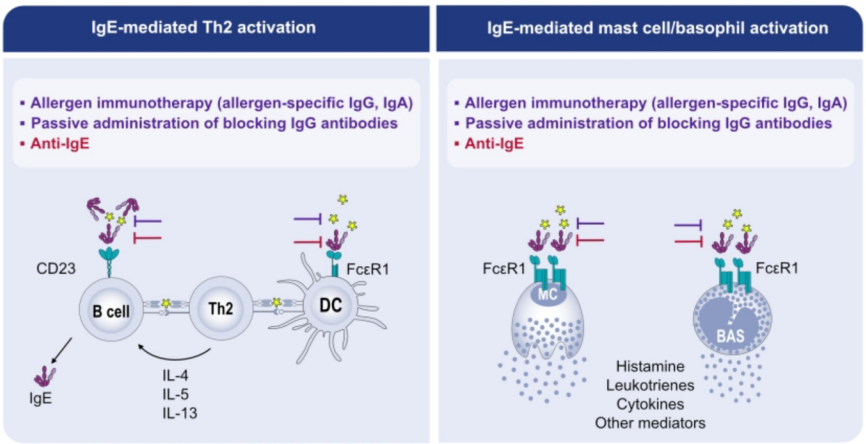

Figure 2 Shamji et al.

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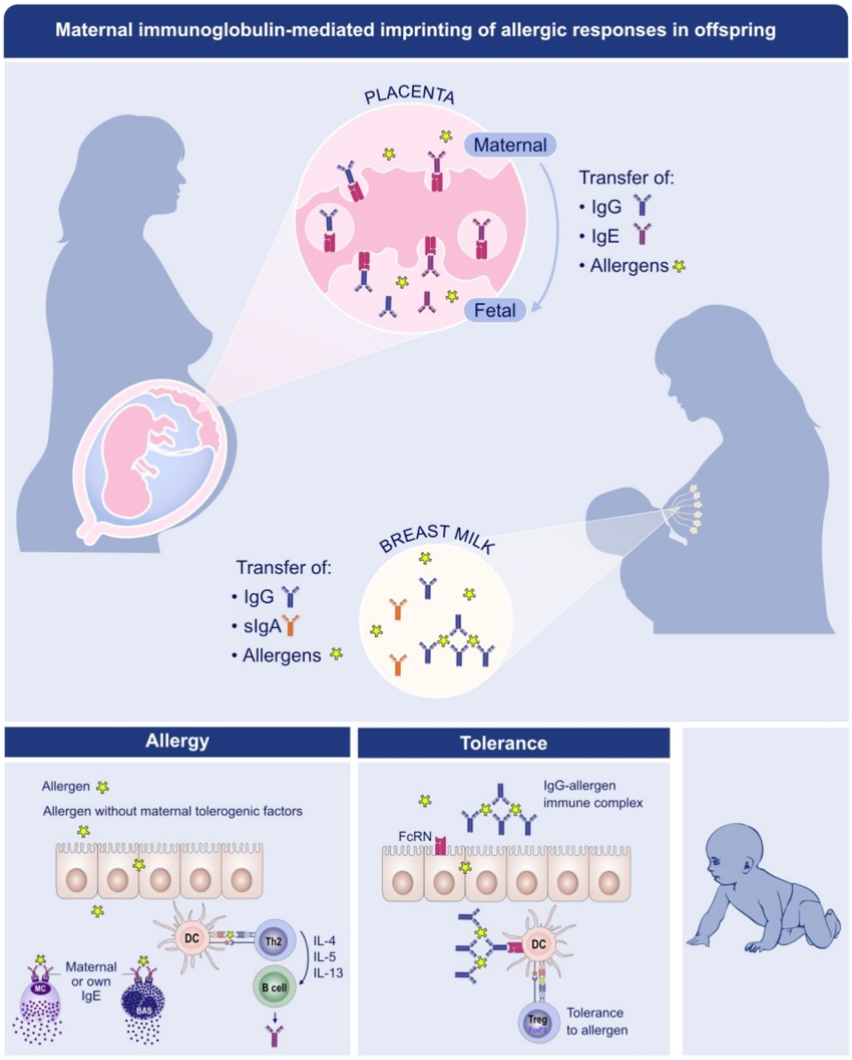

Figure 3 Shamji et al.

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BOX 1: Methods for measuring (effects of) blocking antibodies

Serological assays

The induction of blocking antibodies in allergic patients during AIT can be studied using serological assays demonstrating their ability to inhibit IgE binding to the allergen[114,151]. The allergen neutralising effect of IgG cannot be measured when the amount of allergen on the solid phase is in excess to allergen-specific antibodies as it occurs for example in the ImmunoCAP system (Thermofisher, Uppsala, Sweden)[152]. However, micro-arrays such as the ImmunoCAP ISAC (Thermofisher, Uppsala, Sweden) and the MeDALL allergen chip[153] contain approximately only 100 fg of allergen per spot. Therefore, it is possible to visualise with such assays the competition of IgG with IgE for allergen binding[112,154-156]Therefore, one can compare allergen-specific IgE binding in serum samples obtained before AIT and after AIT, when blocking allergen-specific IgG has developed. In the case that blocking antibodies have developed the IgE signal will be strongly reduced in the post-treatment samples.

Basophils and mast cells

Measuring the effects of blocking antibodies on allergen-induced basophil activation Shortly after developing the allergen-specific basophil histamine release assay, Lichtenstein and colleagues used this test to study the effects of desensitisation during AIT[157]. The effects of blocking antibodies induced by AIT or even of purified human monoclonal allergen-specific IgG antibodies on allergen-induced basophil degranulation can be visualised by pre-incubation of the allergen before exposure to IgE-loaded basophils from allergic patients when the cells had been isolated and washed to remove serum (i.e., basophil activation with washed cells)[158,159]. Alternatively, basophil activation can be performed in blood samples obtained from patients in the presence of serum and blocking antibodies. In this setting, the effects of blocking antibodies become visible due to addition of allergen to the full blood sample containing already the blocking antibodies (i.e., full blood assay)[160].
More recently, rat basophil and mast cell lines transfected with the human $Fc_{\varepsilon}RI$ can be cultivated and loaded with sera obtained before AIT to represent the patient's sensitivity before the treatment. The cells are then exposed to allergen pre-incubated with serum samples obtained before and after treatment to investigate the development of blocking antibodies in the post-treatment serum[108]. These experiments can be performed with sera as such, purified IgG fractions or sera that had been heat-inactivated at 56°C to remove IgE effects. The advantage of using the transfected cell lines is that the experiments can be conducted with all sera simultaneously with cells having comparable sensitivity. In contrast, experiments performed with fresh basophils from patients at different time points can be subject to variations due to general differences of basophil sensitivity occurring in subjects at different time points.

IgE-facilitated allergen presentation

It is known that IgE-facilitated allergen presentation via CD23 on B cells is a key mechanism in allergen presentation to T cells in allergic patients because it allows tiny amounts of allergens to be presented by an efficient pick-up mechanism. This is of particular relevance in allergy because one has to consider that only minute amounts of allergens can enter the systemic circulation of allergic subjects due to the presence of epithelial barriers. The first study investigated if AIT-induced blocking antibodies can inhibit IgE-facilitated allergen presentation via CD23 was published in 1995[113]. In this study, it could be shown that AIT-induced blocking antibodies inhibited allergen-specific T cell proliferation and secretion of inflammatory cytokine responses. This result was remarkable because it indicated that the reduction of T cell activity during AIT is mediated by blocking antibodies and not or not only by T cell-mediated immunological tolerance mechanisms[161]. To simplify the assay, a CD23-expressing B cell line was developed which can be loaded with serum IgE from a patient allergic to the given allergen and one can then measure the binding of labelled allergen and its inhibition by AIT-induced blocking antibodies[115]. Although this FAB assay[115] can be easily performed for extensive screening of sera it has the disadvantage that the cells are usually loaded only with one IgE-containing serum (i.e., indicator serum) and hence one cannot assess the blocking of allergen binding to CD23-bound IgE of each of the patients to be tested. However, one can also perform this assay with APCs obtained from each patient to be tested and add allergen in the presence of pre-and post-treatment sera to measure the development of blocking antibodies in each of the tested patients[160].

A question which still needs to be investigated is if the inhibition of IgE-facilitated allergen presentation and its effects on subsequent T cell activation is related to a certain type of allergic symptoms. By intuition, one would expect that inhibition of T cell activation by blocking antibodies would be related to a reduction of late-phase allergic reactions. However, to study this, it is not sufficient to measure only the effects of blocking antibodies on IgE-facilitated presentation and their effects on T cell activation and relate the latter parameters with clinical effects such as late-phase skin reactions eventually atopy patch test results. It has been challenging to relate allergen-specific T cell proliferation and cytokine secretion in blood-derived cells with atopy patch test results[162]. When comparing serological assays, basophil activation tests and FAB assays a good correlation was observed among the different assays, and it may therefore be sufficient to perform serological assays for the assessment of AIT-induced blocking antibodies[114].

In vivo methods

The classic experiment demonstration that AIT-induced blocking antibodies inhibit allergen-induced skin test reactions by Prausnitz-Kuestner reaction[25,163] in humans[25] cannot be performed any more due to ethical reasons. One, therefore, can only compare results of *in vivo* provocation testing such as skin testing[164,165], conjunctival provocation testing[166] (and allergen exposure testing [160] with the development of potentially blocking non-IgE antibodies in patients during AIT and calculate correlations. A direct demonstration of the effects of blocking antibodies on *in vivo* test results is hence not possible.

Box 1 Shamji et al.