

Review article

Molecular and cellular targets of anti-IgE antibodies

Immunoglobulin E (IgE) was the last of the immunoglobulins discovered. It is present in very low amounts (nano- to micro-gram per ml range) in the serum of normal healthy individuals and normal laboratory mouse strains and has a very short half-life. This contrasts with the other immunoglobulin classes, which are present in much higher concentrations (micro- to milligram per ml range) and form a substantial component of serum proteins. Immunoglobulins play a role in homeostatic mechanisms and they represent the humoral arm of defence against pathogenic organisms. Since IgE antibodies play a key role in allergic disorders, a number of approaches to inhibit IgE antibody production are currently being explored. In the recent past the use of nonanaphylactic, humanized anti-IgE antibodies became a new therapeutic strategy for allergic diseases. The therapeutic rational beyond the idea derives from the ability of the anti-IgE antibodies to bind to the same domains on the IgE molecule that interact with the high-affinity IgE receptor, thereby interfering with the binding of IgE to this receptor without cross-linking the IgE on the receptor (nonanaphylactic anti-IgE antibodies). Treatment with anti-IgE antibodies leads primarily to a decrease in serum IgE levels. As a consequence thereof, the number of high-affinity IgE receptors on mast cells and basophils decreases, leading to a lower excitability of the effector cells reducing the release of inflammatory mediator such as histamine, prostaglandins and leukotrienes. Experimental studies in mice indicate that injection of some monoclonal anti-IgE antibodies also inhibited IgE production *in vivo*. The biological mechanism behind this reduction remains speculative. A possible explanation may be that these antibodies can also interact with membrane bound IgE on B cells, which could interfere the IgE production.

D. Inführ¹, R. Crameri², R. Lamers³, G. Achatz¹

¹Department of Molecular Biology, University of Salzburg, Austria; ²SIAF, Davos, Switzerland; ³MPI for Immunobiology, Freiburg, Germany

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Dr Gernot Achatz
Fachbereich für Molekulare Biologie
Abteilung Allergologie und Immunologie
Hellbrunnerstraße 34
A-5020 Salzburg
Austria

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The biology of IgE

In 1966 Ishizaka et al. (1) opened a new era in the pathophysiology of immunological disorders when they identified and purified IgE from the serum of allergic patients. Like other immunoglobulins, IgE consists of two light chains and two ϵ -heavy chains and can be detected in two forms, a secreted and a membrane-bound form (Fig. 1). mIgE is a transmembrane protein which behaves like a classical antigen receptor on B lymphocytes (2). Previous experiments in our and other laboratories showed that the expression of functional mIgE is essential for generating a humoral IgE and IgG1 response in mice (3, 4). The transmembrane domain and the cytoplasmic tail are encoded by two exons M1 (transmembrane domain) and M2 (cytoplasmic tail). The cytoplasmic domains of mIgs are different in size and range from only three amino acid residues in the case of mIgM and mIgD to 28 residues for the mIg subclasses. The mIg transmembrane segments are about 25 amino acids long, are highly homologous between all Ig-subclasses and have the potential for interaction with other polypeptides (5). Beside these 25 membrane-spanning amino acids, M1

additionally encodes isotype specific extracellular spacer segments. The spacers differ in lengths (13–21 amino acids) and show high variability between the different Ig isotypes. In the early nineties it became evident that human IgE molecules, unlike other immunoglobulin classes, bind specifically and with a very high affinity ($K_a = 10^9$ M) to receptors (Fc ϵ RI) on the surface of human basophils and mast cells (6). IgE cross-linking of Fc ϵ RI⁺ cells by specific antigens results in the release of a variety of preformed (e.g. histamine) and de novo synthesized chemical mediators (e.g. prostaglandins) and cytokines that exert their effects by interacting with specific receptors on target organs. Despite the fact that IgE is known for more than 30 years, we must admit that, so far, we failed to define significant biological functions for the IgE molecule. Because IgE titres are elevated in individuals suffering from helminthic infestations, IgE was thought to play a role in the defence against worms (7, 8). It was surprising to realize that treatment with anti-IgE antibodies of mice infected with *Schistosoma mansoni* or *Nippostrongylus brasiliensis* resulted in accelerated elimination of parasites and in a decreased worm burden and reduction in the number of eggs, which

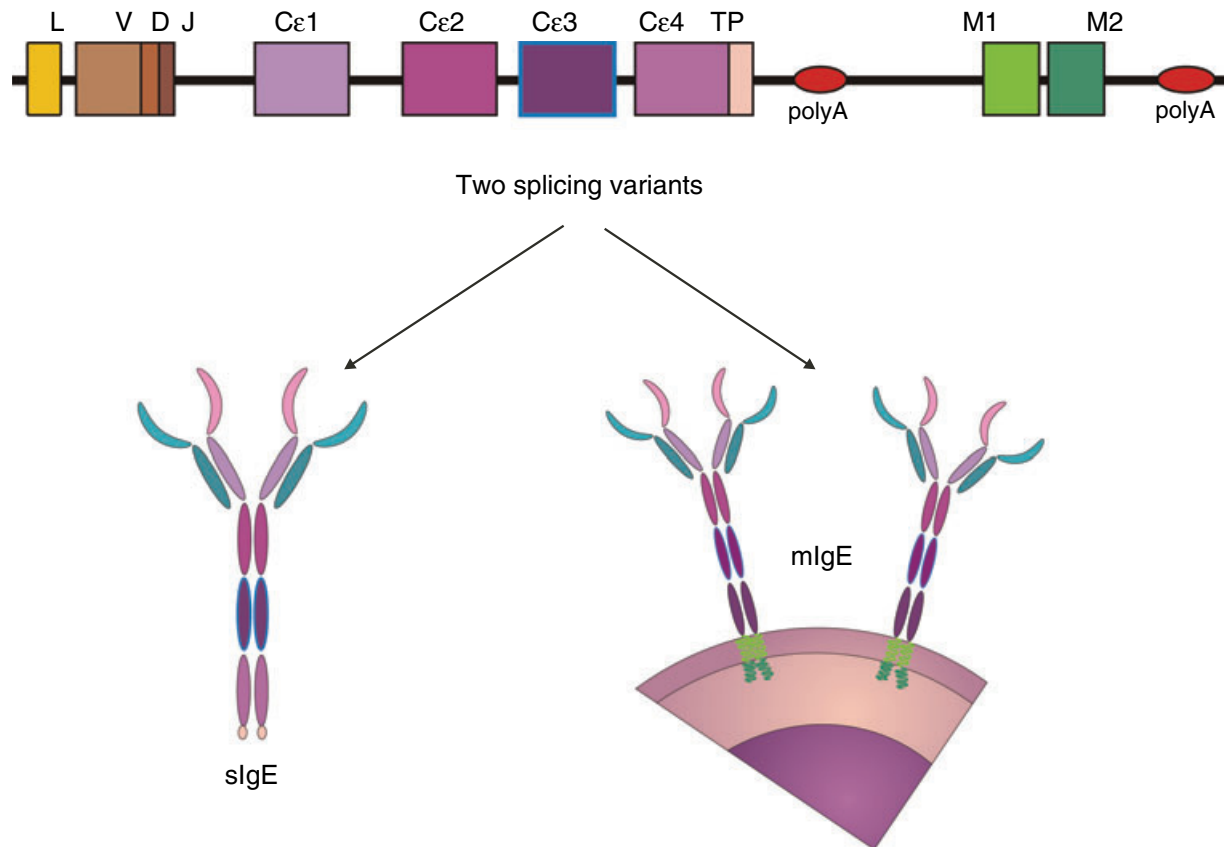


Figure 1. Like all other immunoglobulins, IgE can be found in two forms. In the secreted form (sIg) immunoglobulins represent the effector arm of the humoral immune system. Alternatively, they can also be expressed on the surface of a B lymphocyte in a membrane-bound form (mIg), and, in this physical state, they most likely convey signals to steer the B-cell along its differentiation pathway. The production of the two types are determined by alternative splicing or rather, alternative polyadenylation. The constant parts of the epsilon heavy chains are encoded by six exons. The last two exons code for the transmembrane (M1) and cytoplasmic domain (M2). The fourth exon (CH4) which is located 5' of the membrane exons is a composite exon: it contains an internal splice donor site which is used when mRNA for membrane-bound Ig is made.

paralleled the decrease of serum IgE. High serum IgE levels seem not to be related to host defence but may be the consequence of Th2 cells by the parasite leading to increased IL4-levels. Thus, what remains is the knowledge, that IgE is the key molecule of the allergic response and current drugs for allergic diseases, such as antihistamines, corticosteroids, and bronchodilators, are not able to affect the basic causes of the disease but mainly treat allergic symptoms and concomitant inflammatory reactions. Conventional desensitization immunization with total extracts of allergenic sources (9) often are not effective and go in parallel with anaphylactic reactions. Therefore, a systemic treatment that targets the allergic process, that prevents it from occurring, and has fewer side effects than current drugs, is desirable. Because IgE is the central macromolecular mediator responsible for the progression of allergic reactions, neutralizing it and inhibiting its synthesis would appear to be a rational approach for the treatment of various allergic diseases. However, we must be aware that by systemically blocking the IgE response, we resign on an antibody class without

the knowledge of its biological function and its natural biological function during immune reactions.

The concept of anti-IgE

Several strategies were followed to treat IgE-mediated allergic diseases (Fig. 2) by down-regulating IgE levels (10–13). The basic idea was that chimerized or humanized anti-IgE antibodies with a set of unique binding properties could be used for the isotype-specific control of IgE, and thus would seem a logical therapeutic approach to cure IgE-mediated diseases. The demands for an anti-IgE antibody (Fig. 3) are: the anti-IgE antibody must have a high affinity for IgE, should not bind to IgE already bound by FcεRI on mast cells and basophils, nor to IgE bound by the low-affinity IgE Fc receptors (FcεRII, also known as CD23) on various other cell types and should bind to membrane-bound IgE (mIgE) on mIgE-expressing B cells. Summarizing, these antibodies are designed to neutralize free IgE and to target IgE-expressing B cells (10, 12, 14).

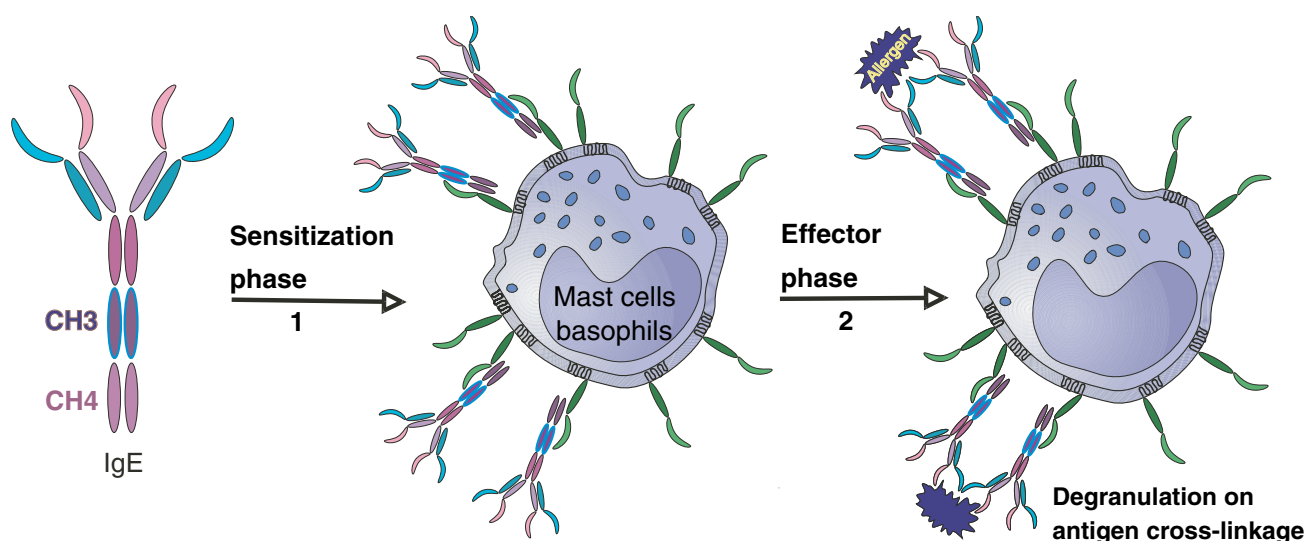


Figure 2. Allergic pathway: The binding of serum IgE to the high affinity IgE receptor on basophilic granulocytes and mast cells initializes the 'allergic pathway' (1). Allergen contact leads to cross linkage of the FcεR1-receptors, resulting in the degranulation of the mast cells and basophils (2).

If these aims are achieved, the levels of IgE in blood and interstitial fluids available for binding to FcεRI will be greatly reduced, and hence the sensitivity of mast cells and basophils to allergens should be gradually reduced. On the other hand, as the anti-IgEs do not bind to IgE bound by FcεRI, they do not crosslink FcεRI-bound IgE and are therefore unable to sensitize mast cells and basophils, in contrast to anaphylactic anti-IgE antibodies (15). Because the envisaged therapeutic anti-IgEs do not bind to IgE bound by FcεRII, which is expressed broadly on lymphocytes, macrophages, platelets, and many other cell types – they are not expected to cause any adverse effects associated with such binding.

Effect of anti-IgE antibodies on free serum IgE

The anti-IgEs under clinical evaluation and development have an association constant, K_a , for soluble IgE of approximately $10^{10}/M$ (16), which is in the range of the affinity of the FcεRI receptor for IgE. Thus, if anti-IgE is maintained at concentrations in excess of IgE in the body, it should effectively compete with FcεRI for IgE. The concentrations of IgE in the blood vary widely among patients with allergy, ranging from 0.05 to 1 µg/ml in most patients. Taking this into account, anti-IgE given in excess to the basal concentration can bind most of IgE, leaving a minimum of free IgE available for binding to IgE Fc-receptors.

Effects on down-regulation of FcεRI

In principle, two kinds of histamine releasing stimuli are known for basophils, namely, an IgE receptor-mediated

and a nonIgE receptor-mediated release. Allergens induce histamine release via FcεRI on the cell surface, and nonIgE receptor-mediated stimuli, such as complements, low-molecular-weight peptides and lipids, induce histamine release via nonIgE receptor-mediated pathways (17). In this connection the most readily appreciated pharmacological effect of anti-IgE therapy is the indirect effect on the down-regulation of FcεRI on basophils. In earlier studies, the density of FcεRI on basophils was found to correlate strongly with the level of IgE in blood. In one of the clinical studies (18) and in additional *in vitro* studies (19), it was found that anti-IgE can also down-regulate FcεRI in patients. The results show that the density of FcεRI on basophils had decreased by more than 95%, and in some cases to more than 99%, after anti-IgE was administered to patients for periods of 5 weeks to 3 month (18). The basophils isolated from patients after anti-IgE treatment were much less sensitive to allergen stimulation. In skin prick tests, it was also found that much larger amounts of allergens were required to induce a positive reaction, indicating that mast cell function was also markedly decreased (18).

Effects on mIgE-bearing B cells

Experimental evidence from *in vitro* and *in vivo* studies is generally supportive of the effectiveness of anti-IgE in targeting IgE-expressing B cells and in inhibiting the continual production of IgE. The idea that anti-IgE can cause these effects is that anti-IgE binds to mIgE on IgE-expressing B cells, and as mIgE is a part of the B-cell receptor, anti-IgE may interfere B-cell signalling or even cause their lysis, like anti-IgM or anti-IgG (20–22). However, IgE-secreting plasma cells do not express mIgE

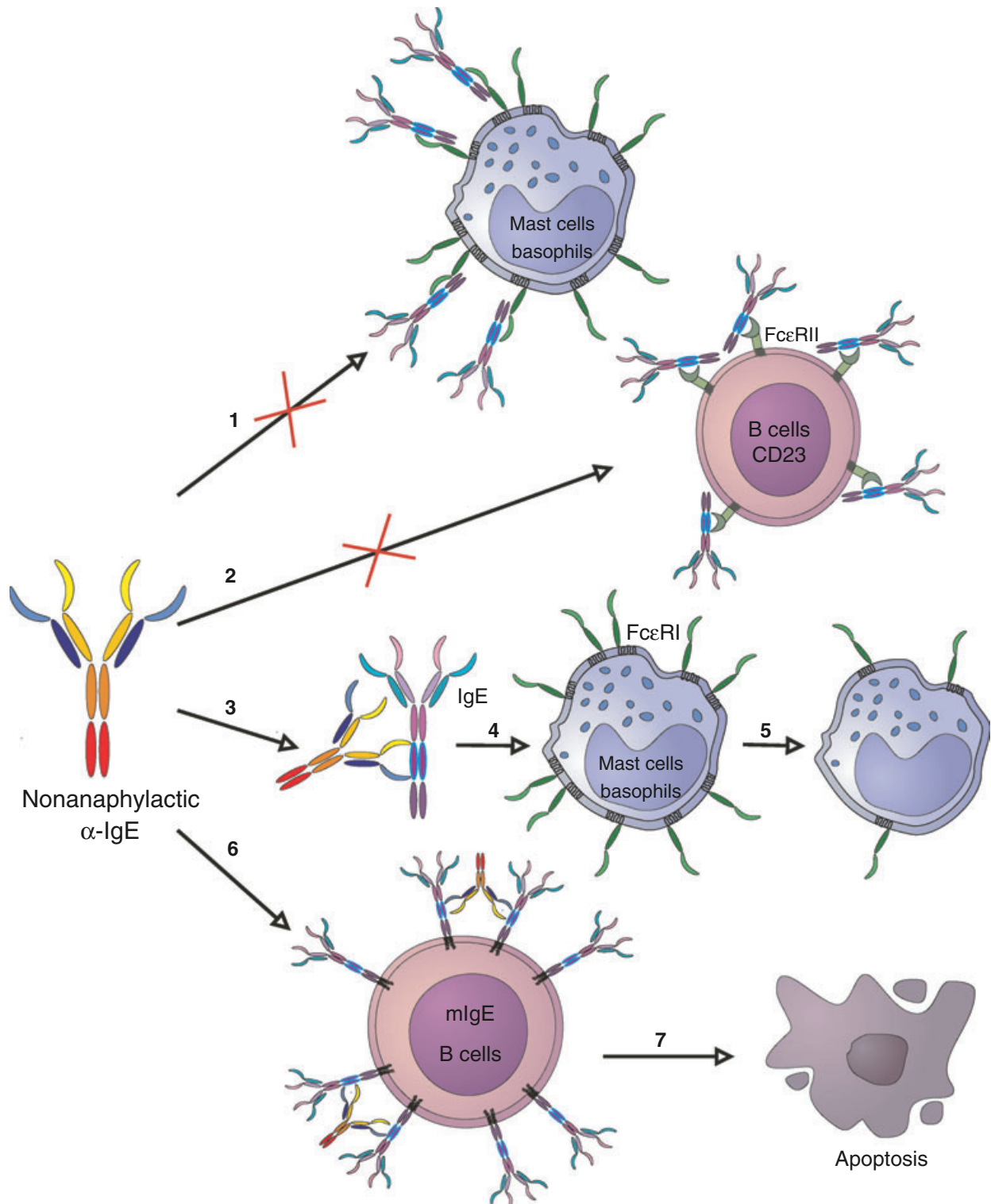


Figure 3. Requirements on nonanaphylactic anti-IgE antibodies are: no activation of sensitized mast cells and basophils (1) and Fc ϵ RII-bound IgE (2) by anti-IgE cross-linkage. Anti-IgE recognizes the CH3-CH4 boundary of free serum IgE (3), thus inhibiting the binding of IgE to the high affinity IgE receptor (Fc ϵ RI) on basophilic granulocytes and mast cells (4). As a result, the number of high affinity receptors, expressed on the surface, decreases (5), leading to a lower excitability of the effector cells. Anti-IgE recognizes the mIgE receptor on B cells (6). The decrease of serum IgE observed may be explained by apoptosis of mIgE expressing B cells, reached by the immunological process of tolerance and/or anergy induction (7).

and presumably are not affected by anti-IgE. These cells reside in the bone marrow and probably have a life span of several weeks to several months. Since new IgE secreting plasma cells go through mIgE-expressing B cell stages during differentiation, if their generation is abrogated by anti-IgE treatment, the existing plasma cells will die off in several weeks to several months, and thus the production of IgE will also gradually abate in similar periods. Furthermore, memory B cells may possibly be affected by anti-IgE. If this occurs, anti-IgE may have long-term effects on the fundamental disease process. The molecular mechanisms, leading to the depletion of these cells can be explained by apoptosis and reached by the immunological process of tolerance and/or anergy induction.

Molecular mechanisms of anergy and/or tolerance induction

The process of tolerance and anergy induction can daily be observed in millions of uniquely different B cells, each with the potential to recognize and eliminate a different invading antigen. But what happens with B cells, which by pure chance rearrange VDJ and VJ combinations recognizing self-structures (23)? The explanation is immune tolerance (24), a natural fail-safe mechanism that eliminates B cells that target 'self,' rather than foreign antigens. Transgenic animal studies confirmed that clonal deletion and clonal anergy are the principal mechanisms of self-tolerance. Clonal deletion is the physical elimination (death) of a B cell, while clonal anergy is the silencing of a B cell so that while it is still alive, it no longer produces antibody, nor can it be readily activated (25). Both mechanisms and/or strategies naturally occur during B-cell development.

It has long been established that the cross-linkage of mIgM on immature B cells (26) by multivalent self-antigen in the bone marrow causes the cells to die (27). This process is called negative selection and leads to central tolerance (26, 28). However, some self-antigens do not have access to the bone marrow. B cells, expressing mIgM specific for such antigens cannot be eliminated by the negative selection process. To avoid autoimmune responses from such self-reactive mature B cells (29, 30), some process for deleting them or rendering them inactive must occur in peripheral lymphoid tissue, a mechanism known as peripheral tolerance (31), finally leading to an unresponsive or anergic state. In today's way of thinking, the B cell binds the antigen via its receptor, which transduces the first signal into the cell via activation of tyrosine kinases, such as syk and lyn (reviewed in (32)). The receptor-antigen complex is then internalized, the antigen processed, and displayed as peptide MHC II complex on the B cell surface. Antigen binding to the B cell also stimulates expression of the co-stimulatory molecules B7-1 and B7-2. If T cells exist with receptors

that can recognize these coreceptor-peptide-MHC complexes, the ensuing T-cell-B-cell interaction provides the second signals in the form of a CD40-ligand CD40 interaction and the release of stimulatory cytokines such as IL4 and IL5 or IFN- γ . In this framework then, antigen stimulation of B cells in the absence of T-cell help leads to tolerance, while stimulation in the presence of antigen specific T-cell help leads to proliferation and differentiation (Fig. 4). In other words, stimulation of B cells via the antigen receptor without appropriate T-cell help normally leads to apoptosis (33). Can these data be adapted for the induction of tolerance and/or anergy of a class-switched mIgE bearing B-cell population by using anti-IgE antibodies? Two scenarios for the functioning of a humanized anti-IgE antibody, which by the immune system is considered as an auto antigen, leading to no recruitment of T cells, would be thinkable. First, as shown by self-reacting immature B cells, cross-linkage of the mIgE receptor without further T-cell support should

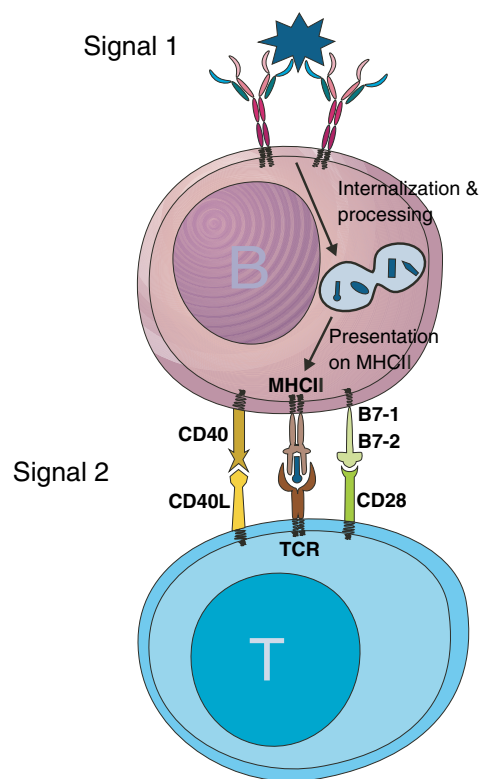


Figure 4. Antigen binding to the B cell receptor transduces the first signal into the B cell through the tyrosine kinases syk and lyn. This leads to activation of RAS and the MAP-kinases as well as phosphorylation to phospholipase γ 1, which results in a rise of intracellular calcium. This signal given alone is proposed to tolerize the cell. Antigen binding to the B cell also stimulates expression of the co-stimulatory molecules B7-1 and B7-2. Internalization and presentation of the peptides on MHC II to T helper cells activates the T cell to express CD40 ligand, which signals the B cell through CD40. When the B cell receives this second signal, it proliferates (52).

directly induce apoptosis. Second, as normally shown during the induction of peripheral tolerance of mature B cells in answer to monovalent (self) antigen, receptor blockage of mIgE by for example a Fab-fragment should result in an anergic state of the mIgE population. In contrast to normal mature B cells, which have a half life of 4–5 weeks, anergic B cells were found to last for only 3–4 days (34). Nevertheless the fate of these anergic B cells finally turns out to be cell death (35).

This simplified scenario is restricted to a very special lymphocyte elimination process. However, T and B lymphocytes undergo apoptosis in many instances in their development, homeostasis, and activation. Lymphocytes die during development if they fail to locate or compete for cytokines, fail to properly rearrange an antigen receptor, fail to be positively selected, or if they are negatively selected. In the periphery, resting lymphocytes are eliminated from or fail to enter the long-lived recirculating pool if they fail to locate or compete extrinsic signals. So for example B cells that bind and present antigenic peptides on MHC II to T cells can also undergo receptor induced apoptosis. Germinal center B cells that lose affinity for selecting antigen or gain autoreactive specificities are also eliminated. Furthermore, lymphocytes that fail to become memory cells after antigen clearance, are also eliminated (reviewed in (36)).

Outlook: anti-mIgE antibodies

Our knowledge about the regulation of the expression of membrane-bound IgE is at best limited. Unfortunately, a similar statement can be made regarding the function of the transmembrane and the cytoplasmic domain of IgE. We do however know that the production of IgE is tightly regulated on the level of DNA recombination (switch), transcription and RNA processing. However, transgenic mouse experiments (3) clearly showed that the transmembrane domain of mIgE is indispensable for T-cell dependent IgE secretion and that the cytoplasmic domain not only determines the absolute amount of IgE produced, but also influences the quality of the immunoglobulins. Thus, if mIgE seems to be the prerequisite for the later production of secreted IgE, targeting mIgE bearing B cells with anti-mIgE-specific antibodies could be a therapeutic possibility. A possible target domain is represented by the extra membrane proximal domain (EMPD), coded as part of the transmembrane domain M1. The EMPD could be used as target sequence for generating anti-mIgE antibodies with the capacity to inhibit IgE synthesis. First results on this approach were published by Chen et al. (37). In this study, the ability of an anti-spacer-specific antibody on targeting and lysing mIgE expressing B cells was examined. Thus, the pharmacological targets are memory B cells involved in secondary immune responses. Summarizing, if anti-mIgE antibodies indeed inhibit or downregulate IgE synthesis

in vivo, these antibodies may be used to treat allergic patients with very high IgE levels. The advantage of this therapeutic approach would be the next step in the generation of anti IgE-therapy, with the advantage of inhibiting IgE secretion before secreted IgE production starts.

Clinical applications of anti-IgE

Summarizing, targeting IgE is a systemic approach against an increased IgE production. Patients suffering from allergic rhinitis are often sensitized to multiple outdoor allergens. For these patients SIT is the only available approach so far. Despite optimized treatment regimens, including antihistamines, corticosteroids and mast cell stabilizers, a subgroup of patients with allergic rhinitis have insufficient symptom control (38). Therefore, new treatment options, like anti-IgE in combination with SIT are desirable to target more specifically and earlier the allergic cascade. Combination therapy may permit a broader use of SIT by reducing the risk of anaphylactic side effects after SIT injections. However, anti-IgE therapy is effective not only for seasonal allergic rhinitis, but has also been successfully tested for allergic bronchial asthma and food allergy. In patients with peanut allergy there is currently no alternative than the avoidance of the allergen itself. The first clinical trial demonstrated that anti-IgE is able to increase the threshold of sensitivity to peanuts (39). The findings of clinical phase II and III trials with anti-IgE antibody treatment in asthmatic patients show that anti-IgE is an effective therapy for moderate to severe allergic asthma (40, 41). It reduces the frequency of exacerbations, improves symptom scores, and reduces the requirements of steroid medication (42). A first study designed to evaluate the efficacy of the anti-IgE antibody omalizumab in 405 randomized patients with concomitant asthma and persistent allergic rhinitis was published by Vignola et al. (43). The clearly outcome was that the anti-IgE antibody was well tolerated and effective in preventing asthma exacerbations and improving quality of life in patients with concomitant asthma and persisting allergic rhinitis (40, 43). Interestingly, IgE receptor-mediated histamine release from basophils is significantly higher in asthmatics than in nonasthmatic controls (17). Kim et al. (44) showed that in asthmatic children a single point mutation in the beta chain of FcεRI (FcεRI-β) enhanced the histamine release from basophils to anti-IgE stimuli. This was the first description of a susceptible single nucleotide polymorphism, affecting FcεRI signalling by enhancing mediator release in response to receptor cross-linking. The results clearly show that anti-IgE is efficacious and safe for the treatment of asthma and further IgE mediated diseases, though further improvements are necessary in the future. Without any limitations, anti-IgE therapy is effective in all age groups that have been studied so far. The optimal

duration of anti-IgE therapy is unknown, but it is likely that anti-IgE has to be administered continuously in a dose dependent fashion. Even if the generation of new IgE producing plasma cells is blocked by anti-IgE, new IgE expressing B cells and thus new plasma cells will be regenerated in a few weeks to a few months time in the absence of anti-IgE. Thus, repeated anti-IgE dosing appears to be necessary. While patients are under anti-IgE treatment, which abbreviates IgE-related immune mechanisms and their manifestations, they remain exposed to the usual allergens. If these allergens drive the immune system toward nonIgE-related responses, the disease process may be gradually attenuated. If anti-IgE can inhibit IgE expressing memory B cells that are responsible for making recurrent IgE responses, the continuous exposure of allergens may preferentially drive the immune system towards the production of antibodies of other subclasses. If anti-IgE can indeed influence a shift in the immune response, the combination of antigen immunotherapy with anti-IgE may present another new approach for individuals suffering from severe allergies.

Th1-inducing adjuvants supporting anti-IgE therapy

Basically, class switch to IgE is induced when B cells interact with Th2 cells in the presence of IL4. These events can be influenced at different levels and a number of strategies have been developed aiming to suppress the Th2 driven class switch recombination. Blocking the IL-4 dependent actions of Th2 cells with antibodies against IL-4 represents another approach using antibodies as a therapeutic tool. From the immunological point of view, this approach will induce neither allergen specific nor long-lasting balancing of allergic responses, and moreover, IL-13 can replace several functional activities of IL-4. In general anti-IL4 and IL-5 treatment has been disappointing and contradictory (45). An alternative was suggested by direct blocking IL-4 and IL-13 synthesis. For this purpose, mutant proteins acting as antagonists were developed (46). With increasing insight into the mechanisms of Th1- and Th2-type immune responses, various possibilities to modulate Th2 immune responses seem possible.

The idea behind is, that Th1-biased stimuli could prevent the development of an allergic response. Bacille Calmette-Guerin (BCG) fulfils the criteria of a safety Th1 induction (47). BCG in combination with allergen induced an anti-allergic effect in animal experiments. Another group of bacilli, the lactic bacteria (LAB) also proved to be anti-allergic candidates (48). LPS represents a ubiquitous molecule of our environment and a number of important immuno-modulatory processes have co-evolved with this molecule. With respect to allergy and asthma, the effect of LPS is still unclear. As an adjuvant co-administered with allergens LPS partly improved the efficiency of SIT without triggering harmful side effects (49). In parallel, stimulation of Th1 inducing cytokines like IFN- γ , IL-12 and IL-18 gave contradictory results in animal experiments (50, 51).

Conclusion

Currently available nonanaphylactogenic anti-IgE antibodies permit interruption of the allergic pathway at an early and central level by efficiently and selectively blocking IgE effector functions mediated by Fc ϵ RI- as well as Fc ϵ RII-bearing effector cells. These antibodies block IgE mediated cell activation and inhibit new IgE production by IgE switched B cells without affecting the production of other antibody classes. Clinical trials in patients with allergic rhinitis and allergic asthma reveal that selective neutralization and inhibition of IgE is associated with the inhibition of IgE mediated reactions. These studies will also encourage further investigations which focus on the selective targeting of mIgE bearing B cells, thus inhibiting IgE synthesis before IgE production starts.

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