Developing antibodies for targeting immunoglobulin and membrane-bound immunoglobulin E

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ABSTRACT

The development of anti–immunoglobulin E (IgE) antibodies for prophylactic and therapeutic applications in allergic diseases has taken nearly 20 years. Limited usage has been approved for allergic asthma. The usage in other allergic indications is in active development and may take an additional 5–10 years. The current therapeutic anti-IgE, which includes two humanized recombinant monoclonal antibodies, is the result of rational drug design, taking into consideration the structural features of IgE and membrane-bound IgE (mIgE) and various techniques collectively referred to as antibody engineering. Unlike ordinary antibodies specific for human IgE, a therapeutic anti-IgE, although capable of binding tightly to free IgE and mIgE, does not bind to IgE bound by the high-affinity IgE.Fc receptors (FcϵRI) on mast cells and basophils and by the low-affinity IgE.Fc receptors (FcϵRII, or CD23) on B cells, granulocytes, and many other cell types. The therapeutic anti-IgE is efficacious in treating allergic patients in whom IgE mediates the major allergic responses. It possesses multiple potential pharmacologic effects; researchers still are uncovering its previously unknown immunoregulatory effects. The human studies of the therapeutic anti-IgE have gradually settled many controversial views relating to IgE, such as the roles of IgE in the pathogenesis of asthma, immune defense, and early and late-phase reactions of allergic responses. Studies on the unique structural mIgE CϵmX domain and on the regulatory effects of anti-CϵmX monoclonal antibodies will be an area for future investigation of the potential of long-term IgE regulation. (Allergy Asthma Proc 27:S7–S14, 2006)

Since the discovery of immunoglobulin E (IgE) as a reaginic class of antibody almost 40 years ago, many applications relating to the diagnosis of allergic diseases and conditions using measurements of total and allergen-specific serum IgE have become well established.[1,2] The development of recombinant anti-IgE monoclonal antibodies (mAbs) for the prophylaxis and therapy of allergic diseases was started in 1987.[3–6] Over 18 years of active development, applications of an anti-IgE antibody for patients ≥12 years of age with moderate-to-severe allergic asthma[7–9] have been approved in several countries. At present, only a very small fraction of patients with allergic asthma have received the new drug.

In various clinical trials, anti-IgE antibodies also have been shown to be efficacious in treating pediatric patients with severe asthma[10,11] as well as patients with seasonal and perennial allergic rhinitis,[12–14] peanut sensitivity,[15,16] and sensitivity toward latex products.[17] As for atopic dermatitis, which is characterized by high blood levels of IgE, the therapeutic effects of anti-IgE still are unclear. In sporadic cases, anti-IgE has been shown to be effective in alleviating atopic dermatitis (meeting reports). Sufficient clinical trials for these indications and their governmental approval processes are estimated to take up to 10 years for the applications to benefit patients in most regions of the world.

The design and production of large amounts of monospecific homogeneous recombinant anti-IgE antibodies are technically feasible, owing to advances in the understanding of IgE's biological and structural properties and the development of major technologies such as hybridoma methodology, antibody display methodologies for screening antibody libraries, recombinant immunoglobulin (Ig) heavy- and light-chain gene construction, Chinese hamster ovary and mouse myeloma line development for expressing transfected Ig genes, and the large-scale culture of producer cell lines in bioreactor tanks. The numerous techniques applicable for making improved antibodies for human *in vivo* therapeutic and diagnostic uses are collectively referred to as antibody engineering technology.[18,19]

The pharmaceutical program of anti-IgE evolved at a time when controversies in the IgE field abound. Whether IgE was involved in the pathogenesis of asthma was in debate during the 1980s, and the best evidence then was the finding that IgE was correlated with the severity of asthma in certain regional clinical investigations. Whether IgE plays an essential immune defense role or whether it could be compromised was unclear. Because the relationship between the early and late-phase reactions in allergic diseases was very vague, whether targeting IgE represents a sensible approach was considered dubious by many leaders in the

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This research is supported by a grant, #94-2320-B007-004, from the National Science Council, Taiwan
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allergy and immunology field. The continual human studies of anti-IgE have helped to clarify these controversies.

When the author of this article introduced the anti-IgE therapeutic concept, he envisioned a set of pharmacologic mechanisms by which the anti-IgE mAbs could work to control IgE and alleviate allergic symptoms.[3,4] As anti-IgE mAbs were investigated during the clinical trials, many additional interesting pharmacologic and immunoregulatory properties of anti-IgE, which contribute to the therapeutic efficacy of anti-IgE, were unraveled. Indeed, previously unknown effects of anti-IgE are continually being found and suggested.

This article addresses the aforementioned aspects of the anti-IgE development and discusses the direction of future research relating to the application of anti-IgE mAbs. It also discusses the discovery of a unique structural domain of 52 amino acid residues, CεmX, on membrane-bound IgE (mIgE), and current efforts exploring the potential of using anti-CεmX mAbs to target $mIgE^+$ lymphoblasts and memory B cells in the IgE-mediated allergic pathway.[20,21]

MAJOR CONCEPTS SURROUNDING THE ANTI-IgE INVENTION

This section briefly recounts the concepts, some of which were contentious or in opposition during the early phase of the anti-IgE program from the late 1980s to the early 1990s. During this period, most of the pharmaceutical sectors involved in asthma and allergy adopted approaches that either target the inflammation process and the pharmacologic mediators released by mast cells and basophils or aim at relieving the bronchial constriction. As such, the anti-IgE concept differs dramatically from these contemporary approaches. Furthermore, the roles of IgE in immune defense and in the pathogenesis of allergic responses were not fully understood. Hence, there was widespread skepticism of the anti-IgE approach in the initial years of the program. Fortunately, officials and scientists at the U.S. Food and Drug Administration (FDA) and at the U.K. Medicines Control Agency (now reorganized) welcomed this novel approach with cautious interest and provided guidance in helping us prepare for the first human experimentation. The anti-IgE antibody was first administered to patients in Southampton, United Kingdom, in 1991.[22,23] Some of the contentious issues relating to IgE are highlighted in the following sections.

Is IgE Dispensable?

IgE had been identified as a class of antibodies that had an origin common in many animal species. Although IgE had been found to be reaginic and laboratory findings about IgE had been nearly all related to its allergenic properties, the general belief was that IgE plays an important immune defense role. The findings that IgE was elevated among patients infected with parasites[24] and that in the infected patients, parasitic larva were coated by IgE and attacked by an army of activated eosinophils[25] indicated an IgE role in the immune defense against parasites.[26] On the other hand, it also was known that in people, the blood concentrations of IgE, unlike those of other components, vary over a very broad range of >1000-fold. In some healthy people, IgE is nearly undetectable. Mice, which were deficient in IgE because of a null mutation in the Cε gene or by the treatment of anti–interleukin-4 or anti–interleukin-5 antibodies or other means could mount effective immune responses against parasite challenges.[27,28] These facts suggest that IgE probably played important immune defense roles during the evolution of many animal species, but immune defense functions are highly redundant, and IgE seems to be dispensable, at least under certain environments. It should be noted that most of the references cited in the foregoing discussion are relatively recent, indicating that much of the controversy about the essential role of IgE in parasite immunity still exists.

Is IgE Involved in the Pathogenesis of Asthma?

Although the monitoring of antigen-skin-prick test reactivity and allergen-specific IgE levels at different disease states gradually established the role of IgE in the pathogenesis of allergic rhinitis, the role of IgE in asthma was much more ambiguous. Immunopharmacologists studied mast cells and their mediators and their roles in inflammation and in diseases such as asthma, even before they knew of IgE.[29] Many physicians treating asthma patients regarded asthma as a chronic inflammatory disease and used medications, predominantly corticosteroids and β2-agonists, which alleviate inflammatory processes and relax bronchial constriction. On a practicality basis, these doctors had no strong reason to relate asthma to allergy and had even less of a reason to consider a possible role of IgE in asthma.

On the other hand, the emerging facts that mast cells and basophils, which are packed with granules filled with inflammatory mediators, express the high-affinity receptors of IgE.Fc,[30] and that many known environmental allergens, which caused asthma attacks, can sensitize basophils and mast cells,[31] persuaded some physicians that "allergic asthma" may have a pathological component that is IgE related. A few groups actively investigated the levels of serum IgE in patients with asthma and other types of allergic diseases in certain geographical regions and found that the levels of total serum IgE were higher in patients with asthma than in normal subjects.[32,33] Although these findings

did not establish a direct involvement of IgE in the pathogenesis of asthma, they were, nevertheless, suggestive.

Is IgE a Molecular Target Suitable for Therapeutic Intervention?

When the anti-IgE approach first emerged in the late 1980s, some scientists agreed that IgE seems a logical target for therapeutic intervention, while others held opposite opinions. Even among those who appreciated that IgE is a central mediator of the IgE allergic pathway leading to cascades of inflammatory processes in some allergic indications, many reasoned that screening for small molecules or designing small molecules based on the structure of FcεRI, which can interfere with the binding of IgE to FcεRI on mast cells and basophils, was the only sensible approach.[34,35] Indeed, several large pharmaceutical companies were screening their chemical libraries for candidates that could inhibit IgE binding to recombinant FcεRI. Developing an anti-IgE mAb, in which its administration would likely require injection, simply did not appeal to most academicians and clinicians in the allergy field.

Some of those who disagreed that IgE is a logical step for therapeutic intervention worried that even though the new anti-IgE had been designed not to bind to IgE on mast cells and basophils, it might still bind to IgE on those cells and sensitize them and cause anaphylaxis in rare patients. Some reasoned that IgE could not be sufficiently inhibited or neutralized to achieve a pharmacologic effect. They referred to certain well-known but unpublished studies, which indicated that IgE is bound by FcεRI for extended periods. In those experiments, mice were injected subcutaneously with IgE specific for trinitrophenol (TNP; a hapten often used to study antigen–antibody interaction). After >1 year, when the mice were given a TNP-conjugated protein intravenously, they still could develop inflammatory reactions at the skin site where TNP-specific IgE was injected. These results suggested that mast cells stayed at the injection site and retained some bound TNP-specific IgE for more than a year. Therefore, it was argued that even if anti-IgE could neutralize almost all, say 99%, of the free IgE and inhibit substantially the new synthesis of IgE, the IgE remaining bound to mast cells still could lead to allergic responses.

The Advent in Developing mAb-Based Therapeutics

The author of this article conceived the anti-IgE therapeutic idea and launched a research program to produce the desired mAbs in 1987 in a new laboratory in Tanox, a company he cofounded a year earlier. Before Tanox, he had worked in research groups in Ortho Pharmaceutical Corp. (a subsidiary of Johnson and Johnson Co.) and Centocor Corp., who had pioneered the making and application of mAbs, particularly, those specific for human cellular surface antigens. While at Ortho, he worked on the biological properties of OKT3, a mouse mAb, and helped establish that the antigen OKT3 recognizes is a component associated with the T-cell receptor (before the T-cell receptor was identified).[36] Owing to the joint efforts of Ortho and some academic groups, OKT3, which was later shown to bind to the ε-component of the T-cell receptor, went through clinical trials[37] and was approved in 1986 by the U.S. Food and Drug Administration for use as an immunosuppressive agent to inhibit the rejection reaction in kidney transplantation. OKT3 was the first mAb approved for marketing. In the human clinical studies, it was found that OKT3 causes a rapid disappearance of T cells from the blood circulation within hours and a gradual reduction of T cells in the lymphonodes over a period of several days.[37,38] The experience working with OKT3 and witnessing its successful product development influenced the author in conceptualizing the anti-IgE therapeutic idea. A major theme in his research endeavor was to search for an antigenic site on the B-cell receptor on IgE-expressing B cells that would be suitable for immunologic targeting. Moreover, as mentioned earlier, the rapid advent in many related technologies that can improve mouse antibodies for use in humans helped advance the anti-IgE program.

THE STRUCTURAL AND BIOLOGICAL PROPERTIES OF ANTI-IgE

In the late 1970s and early 1980s, researchers were using anti-IgE polyclonal antibodies, which could induce the cross-linking of IgE bound by the high-affinity IgE receptors, to study the sensitization of basophils isolated from patients or of a basophilic cell line under various conditions.[39,40] As the newly invented hybridoma methodology became more popular, some scientists developed mouse mAbs specific for human IgE and used them in place of polyclonal anti-IgE antibodies. Later, scientists began to ask whether anti-IgE mAbs could behave differently in their abilities to sensitize basophils.[41] A few research groups also studied the mechanisms by which IgE production may be regulated. Nisonoff's group discovered that by administering neonatal mice with IgE, the production of IgE and the number of IgE-secreting plasma cells may be reduced.[42] Other groups reported that in allergic patients and normal people, IgG antibodies against the idiotypic and isotypic regions of IgE are present and that these antibodies may be involved in regulating IgE production.[43]

The Structural Features of an Anti-IgE Antibody

Anti-IgE mAbs, such as Omalizumab and TNX901, both of which have been studied in patients, are hu-

manized IgG antibodies. Originally, they were developed as mouse antibodies through the hybridoma methodology.[3] Using a combination of antibody engineering tools, the genes of the heavy (H) and light (L) chains were substantially reconstructed so that the antigen-binding segments, known as hypervariable regions or complementarity-determining regions, in the variable domains of the H and L chains (V_H and V_L domains) were retained and essentially all framework segments in V_H and V_L were replaced by homologous human ones chosen from the most homologous human H- and L-chain sequences in the antibody database. All of the constant regions of the H- and L-chain genes were replaced by corresponding constant regions of human γ1- and κ-chains. Because the complementarity-determining regions are highly variable among individual antibodies,[5,44] Omalizumab and TNX901 essentially are the same as authentic human IgG1 antibodies in structure, immune effector functions, and antigenicity. The completed recombinant H- and L-chain genes were transfected into and expressed by a mammalian cell host, Chinese hamster ovary cells, which were grown in smartly engineered large bioreactor tanks.

The anti-IgE humanized IgG1 antibody is a protein with a molecular weight of ~150,000 Da. In humans, it has a half-life of ~20 days,[23,45] whereas IgE has a much shorter half-life of 1–2 days. The clearance rate of injected anti-IgE depends partly on the IgE concentration in the patients. Omalizumab is administered by subcutaneous injection at a dosage of 150–400 mg per treatment every 2 or 4 weeks. Currently, it is provided as powder and reconstituted with a buffer at the time of use. A liquid formulation ready for injection is being developed.

The Binding Properties of an Anti-IgE

The power of mAb technology is that antibodies specific for certain functional regions or sites of a protein molecule, such as IgE, can be generated and produced in large quantities. Because the anti-IgE antibody was originally designed to possess a set of required binding specificities, the selected candidates had these binding specificities.[3,4,6] After the anti-IgE antibodies had undergone a series of human studies, additional binding properties have been identified. The following known binding properties of an anti-IgE antibody are listed:

1. It binds to free IgE in the blood and interstitial space, with a high affinity (K_d is in the range of $2–5 \times 10^{-10}$). It does not bind to other substances in the blood and body fluids.
2. It binds to mIgE on B cells. It does not bind to other molecules on B cells or to other cell types. The B-cell receptor containing mIgE is known to express on IgE-expressing B lymphoblasts and memory B cells.
3. It does not bind to IgE bound by high-affinity FcϵRI receptors on the surface of basophils and mast cells. It does not sensitize basophils and mast cells.
4. It does not bind to IgE bound by low-affinity FcϵRII receptors or CD23 on the surface of B cells, granulocytes, platelets, or many other cell types.
5. By binding to IgE, it inhibits the binding of IgE to FcϵRI and FcϵRII.
6. It forms complexes with IgE that are no larger than a 3 anti-IgE:3 IgE hexamer.[22,46] The anti-IgE:IgE complexes can bind to allergen molecules.

The fact that IgE exists in minute concentrations in the blood and forms small soluble complexes with anti-IgE probably help anti-IgE not to elicit antibody response and not to cause immune complex diseases in the treated patients. If anti-IgE could induce antibody response and/or form large immune complexes, the consequence would be detrimental and such anti-IgE would not be suitable for therapeutic use. The question why anti-IgE molecules, which have two antigen-binding arms, and IgE molecules, which have two epitopes for anti-IgE to bind to, do not form large lattices still is not understood. Nature may contribute to another key feature: the binding sites on IgE for FcϵRI and FcϵRII, which are structurally unrelated, are spatially very close or overlapping, allowing[1] the same anti-IgE antibody to block IgE binding by both receptors and[2] both receptors to block IgE binding by the same anti-IgE antibody.

Potential Multiple Pharmacologic Effects of Anti-IgE

Because anti-IgE has been shown to be efficacious in alleviating allergic symptoms in appropriate patients with various allergic diseases, immunologists and clinicians are trying to understand how anti-IgE works under various conditions. Some of the potential pharmacologic properties were unforeseen at the time of the anti-IgE invention. Perhaps the most striking is that the depletion of free IgE by anti-IgE causes the gradual down-regulation of FcϵRI on basophils and mast cells[47–49] and dendritic cells.[50] Since down-regulation of FcϵRI may take a few weeks to a few months to become pharmacologically significant and anti-IgE appears to show effects in some patients in 1 or 2 weeks, it is likely that additional pharmacologic effects play roles at different time frames and in different patients.[6] Figure 1 illustrates the potential multiple drug effects of anti-IgE in the IgE-mediated allergic pathway, as follows:

1. Anti-IgE binds to free IgE and blocks its binding to FcϵRI.

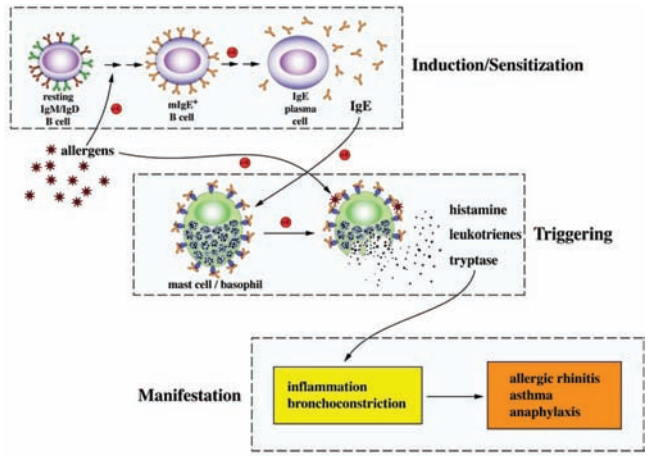

Figure 1. The known and potential multiple pharmacologic and immunoregulatory effects of anti-IgE on the IgE-mediated allergic pathway. These effects are explained in the text. Note that these sites of drug action are the early steps in the IgE-mediated allergic pathway.

2. The depletion of free IgE causes down-regulation of FcεRI on basophils and mast cells over a period of a few weeks to a few months, rendering these inflammatory cells insensitive to stimulation by allergens.[47–49]
3. Anti-IgE binds to IgE, forming small complexes, which accumulate 1–2 weeks after the first anti-IgE injection to levels that are about 10 times the basal IgE levels in the blood.[23,46] These immune complexes could bind to incoming allergen molecules, preventing their access to FcεRI on basophils and mast cells.[6]
4. Anti-IgE binds to the mIgE on B cells. The mIgE is part of the B-cell antigen receptors of IgE-expressing B lymphoblasts and memory cells.[3,4,6] *In vitro* and animal model studies show that the B cells are inhibited or lysed, and the productions of ε messenger RNA (mRNA) and IgE protein are reduced.[4] The effect of anti-IgE on IgE synthesis in treated patients still is not clear. This is partly because the IgE-secreting plasma cells, which do not have very low levels of mIgE on the surface and are not targets of anti-IgE, have very long life spans and continuously secrete IgE, masking the potential effect (if existing) of anti-IgE on lymphoblasts and memory cells.[6]
5. The allergen–IgE complexes, which normally bind to FcεRII (CD23) on B cells and drive IgE-biased immune responses,[51] have become nonexistent.
6. The depletion of free IgE causes down-regulation of FcεRI on dendritic cells, which is evident in 2 weeks[50]; this may divert the immune system away from making IgE-favored responses.

It is clear that all of these potential pharmacologic effects work in the early part of the IgE-mediated allergic pathway, unlike most of the current medicines (aside from allergen-specific immunotherapy [SIT]), which target[1] inflammatory mediators toward the end of the IgE pathway or[2] manifested symptoms.

FUTURE RESEARCH ON TARGETING THE IgE PATHWAY

As discussed in earlier sections, developing the application of anti-IgE to treat other allergic diseases such as pediatric asthma, allergic rhinitis, food allergy, and perhaps allergic reactions to drugs, latex products in most major regions of the world will take many years. The development of an anti-IgE of ultrahigh affinity, which may potentially be used at smaller dosages than anti-IgE, also will likely be an area using research and development resources. Other than these directions, the following are the other areas that warrant vigorous efforts.

Use of Anti-IgE to Prime Allergic Patients for Allergen SIT

Many allergists have been trained to apply SIT (allergy shots) to treat patients with severe allergic problems. In practicing the use of SIT, a large number of allergists have developed a sophisticated knowledge base in diagnosing and treating patients who are allergic to certain environmental allergens in the local areas they live or work. Although SIT may not work for all patients, about one-half of the patients receiving SIT respond well or partially and, in fact, some of them are cured.

The shortcomings of SIT are that (a) it usually requires many months or even many years of repeated injections to produce some appreciable effects in the treated patients and (b) it occasionally inadvertently induces anaphylactic reactions, as the allergens are administered to the patients. Thus, an idea currently pursued by several leading allergy researchers is to explore the potential application of anti-IgE to prime patients for more vigorous and safe SIT (see the article by T. Casale in this volume; also see Kuehr et al. 2002,[52] Rolinck-Werningaus et al. 2004,[53] and Hamelmann et al. 2003[54]). Because anti-IgE can reduce immediate-type hypersensitivity reaction (a major mechanism of anaphylactic reactions) in patients toward administered allergens, anti-IgE can potentially minimize the anaphylactic reactions induced by allergen injections and allow patients to receive much-accelerated SIT, even immunizations-compacted rush SIT. Thus, the combination of anti-IgE with SIT will still use allergists' vast knowledge base in applying SIT and potentially help patients to substantially shorten their treatment protocols and achieve a cure at increased rates.

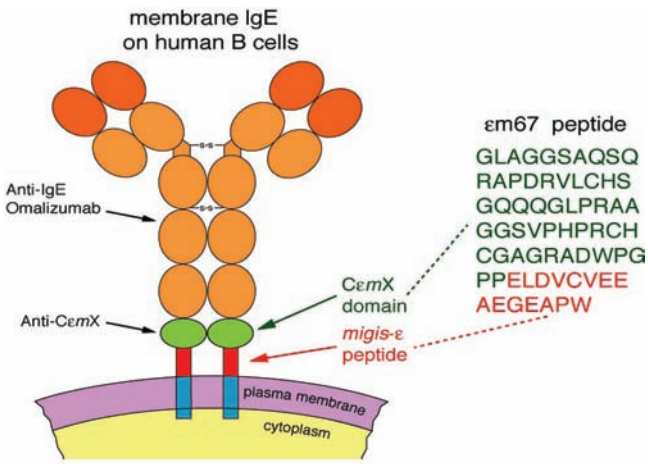

Figure 2. The locations in the mϵ-chain and the a.a. sequences of CϵmX domain and the migis-ϵ-peptide. The term em67 is used to refer to a peptide segment that contains the consecutive CϵmX domain and migis-ϵ-segment.

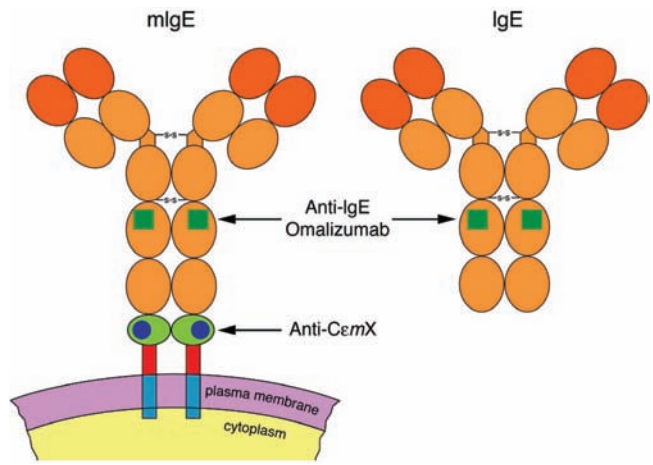

Figure 3. A schematic illustration showing the structural difference between free IgE and mIgE on B cells and the antigenic sites to which anti-IgE and anti-CϵmX antibodies bind.

The Unique CϵmX Domain on mIgE on B Cells

The author's research group discovered in 1990 that the membrane ϵ-chain (mϵ) of mIgE on human B cells contains an extra domain of 52 amino acid (a.a.) residues, referred to as the CϵmX domain, located between the CH4 domain and the C-terminal membrane-anchoring peptide[20] (Fig. 2). In reference to the structure of mϵ-chain known previously from studies on mouse mIgE, CϵmX in human mϵ is a consequence of an alternative splicing of the first membrane exon during RNA processing of the ϵ-transcript. In human and several primate species studied, the mϵ is composed of predominantly the long form containing CϵmX and very little or no regular form; in other animals such as mice, rats, dogs, cats, and horses, mIgE does not contain CϵmX (unpublished results, T. W. Chang, 2003).

In addition, the segment of the first 15 a.a., referred to as *migis*-ϵ-peptide (denoting the mIg isotype-specific peptide of the ϵ-chain), of the C-terminal anchor peptide of mϵ is exposed on the exterior surface of the plasma membrane of mIgE-expressing B cells.[55] The 67-a.a. sequence of the consecutive peptide span of CϵmX and *migis*-ϵ, referred to as em67, is unique in the protein database (Fig. 2). In the IgE production pathway, the B lymphoblasts on their way of maturation to IgE-secreting plasma cells and the IgE-expressing memory B cells express mIgE. Thus, em67 represents a unique site for immunologic targeting of mIgE-expressing B cells for regulating IgE production.

The development of the anti-IgE program has been a priority in the author's research efforts over the past 20 years. However, his laboratory has developed a mouse mAb against CϵmX,[21] which is in the process of being humanized. Efforts also have been made to solve its three-dimensional structure and to develop a rodent model to investigate CϵmX. These studies may help understand the function of this extraordinary domain and develop anti-CϵmX as an immunologic agent to regulate IgE. It is possible that much smaller amounts of anti-CϵmX, as compared with those required to neutralize free IgE, may be sufficient to target and affect IgE-expressing B cells. Potentially, anti-CϵmX may be used in combination with anti-IgE to control and regulate the production and activity of IgE (Fig. 3).

Human Studies with Anti-IgE Help Us Understand the Allergic Pathway

The anti-IgE therapeutic has provided a treatment option for allergic asthma for adolescent and adult patients in some countries. It also may be developed in future years as a treatment option for other allergic diseases for patients living in many regions of the world. However, anti-IgE is not ideal partly because of its high manufacturing costs; therefore, scientists will continue to invent and develop other therapeutic approaches for treating allergic diseases. The continual emergence of new treatment modalities for allergic diseases is propelled by our better understanding of allergic responses and the pathogenesis of various allergic diseases. Certainly, past and ongoing human clinical studies of anti-IgE have contributed to our understanding of the allergic pathways and of the pathogenesis of various allergic diseases.

As discussed in earlier sections, when the anti-IgE program first emerged in the late 1980s and early 1990s, there were controversial views on several pertinent aspects relating to the IgE-mediated immune responses and allergic pathway. Although we have now gained insight in some of those aspects through the *in vivo* human studies with anti-IgE, many issues still are under investigation and not fully resolved. These issues include the following:

1. The role of IgE in immune defense and immune surveillance and whether IgE is dispensable.
2. The relative kinetics of (a) the FcεRI decrease and (b) the loss of sensitivity to allergen stimulation of mast cells on the anti-IgE administration.
3. Whether anti-IgE, by its ability to (a) decrease FcεRI on mast cells and dendritic cells and (b) divert allergen–IgE complexes from binding to FcεRII, attenuates the allergen-induced antigen presentation process that favors IgE production,
4. The role of IgE in the pathogenesis of allergic asthma,[56] the difference in the pathology of asthma in different patients, and how to predict the responses to anti-IgE treatment among asthma patients.[57,58]
5. The role of IgE in late-phase reactions[59] and in chronic inflammation in asthma and in other allergic diseases.
6. Whether IgE is a main mediator in most cases of allergic rhinitis, and what are the allergic mechanisms in those cases in which IgE is not a main mediator?
7. Whether IgE is a main mediator of allergy to peanuts and other foods[60] and whether targeting IgE by a therapeutic-like anti-IgE can alleviate such allergy.
8. Whether IgE is involved in some cases of drug allergy and whether such allergy can be treated by targeting IgE.
9. To what extent IgE is involved in the pathogenesis of latex sensitivity and whether most cases of such allergy can be alleviated by neutralizing IgE.
10. To what extent IgE is involved in some cases of atopic dermatitis.

In the future, more researchers and clinicians will be able to use anti-IgE to investigate these issues and get a better understanding of and treatment options for various allergic diseases.

REFERENCES

1. Yunginger JW, Ahlstedt S, Eggleston PA, et al. Quantitative IgE antibody assays in allergic diseases. J Allergy Clin Immunol 105:1077–1084, 2000.
2. Johansson SG. ImmunoCAP Specific IgE test: An objective tool for research and routine allergy diagnosis. Expert Rev Mol Diagn 4:273–279, 2004.
3. Chang TW, Davis FM, Sun NC, et al. Monoclonal antibodies specific for human IgE-producing B cells: A potential therapeutic for IgE-mediated allergic diseases. Biotechnology (NY) 8:122–126, 1990.
4. Davis FM, Gossett LA, Pinkston KL, et al. Can anti-IgE be used to treat allergy? Springer Semin Immunopathol 15:51–73, 1993.
5. Presta LG, Lahr SJ, Shields RL, et al. Humanization of an antibody directed against IgE. J Immunol 151:2623–2632, 1993.
6. Chang TW. The pharmacological basis of anti-IgE therapy. Nat Biotechnol 18:157–162, 2000.
7. Lanier BQ, Corren J, Lumry W, et al. Omalizumab is effective in the long-term control of severe allergic asthma. Ann Allergy Asthma Immunol 91:154–159, 2003.
8. Busse W, Corren J, Lanier BQ, et al. Omalizumab, anti-IgE recombinant humanized monoclonal antibody, for the treatment of severe allergic asthma. J Allergy Clin Immunol 108: 184–190, 2001.
9. Holgate ST, Chuchalin AG, Hebert J, et al. Omalizumab 011 International Study Group. Efficacy and safety of a recombinant anti-immunoglobulin E antibody (omalizumab) in severe allergic asthma. Clin Exp Allergy 34:632–638, 2004.
10. Berger W, Gupta N, McAlary M, et al. Evaluation of long-term safety of the anti-IgE antibody, omalizumab, in children with allergic asthma. Ann Allergy Asthma Immunol 91:182–188, 2003.
11. Milgrom H. Anti-IgE therapy in children with asthma. Minerva Pediatr 56:469–479, 2004.
12. Adelroth E, Rak S, Haahtela T, et al. Recombinant humanized mAb-E25, an anti-IgE mAb, in birch pollen-induced seasonal allergic rhinitis. J Allergy Clin Immunol 106:253–259, 2000.
13. Nayak A, Casale T, Miller SD, et al. Tolerability of retreatment with omalizumab, a recombinant humanized monoclonal anti-IgE antibody, during a second ragweed pollen season in patients with seasonal allergic rhinitis. Allergy Asthma Proc 24: 323–329, 2003.
14. Chervinsky P, Casale T, Townley R, et al. Omalizumab, an anti-IgE antibody, in the treatment of adults and adolescents with perennial allergic rhinitis. Ann Allergy Asthma Immunol 91:160–167, 2003.
15. Leung DY, Sampson HA, Yunginger JW, et al. Longitudinal Study of Parents and Children Study Team. Effect of anti-IgE therapy in patients with peanut allergy. N Engl J Med 348:986–993, 2003.
16. Leung DY, Shanahan WR Jr, Li XM, et al. New approaches for the treatment of anaphylaxis. Novartis Found Symp 257:248–260, 2004.
17. Leynadier F, Doudou O, Gaouar H, et al. Effect of omalizumab in health care workers with occupational latex allergy. J Allergy Clin Immunol 113:360–361, 2004.
18. Maynard J, and Georgiou G. Antibody engineering. Annu Rev Biomed Eng 2:339–376, 2000.
19. Hutchings C, Carmen S, and Lennard S. Generation of naive human antibody libraries. In Antibody Engineering. Kontermann R, and Dubel S (Eds.) Berlin: Springer Laboratory Manuals, 93–108, 2001.
20. Peng C, Davis FM, Sun LK, et al. A new isoform of human membrane-bound IgE. J Immunol 148:129–136, 1992.
21. Chen HY, Liu FT, Hou CM, et al. Monoclonal antibodies against the CεmX domain of human membrane-bound IgE and their potential use for targeting IgE-expressing B cells. Int Arch Allergy Immunol 128:315–324, 2002.
22. Corne J, Djukanovic R, Thomas L, et al. The effect of intravenous administration of a chimeric anti-IgE antibody on serum IgE levels in atopic subjects: Efficacy, safety, and pharmacokinetics. J Clin Invest 99:879–887, 1997.
23. Racine-Poon A, Botta L, Chang TW, et al. Clinical efficacy of CGP 51901, an anti-IgE chimeric monoclonal antibody, in patients with allergic rhinitis: Is it related to the extent of IgE suppression and pharmacokinetics? Clin Pharmcol Ther 62:675–690, 1997.
24. Rihet P, Demeure CE, Bourgois A, et al. Evidence for an association between human resistance to *Schistosoma mansoni* and high anti-larval IgE levels. Eur J Immunol 21:2679–2686, 1991.
25. Gounni AS, Lamkhioued B, Ochiai K, et al. High-affinity IgE receptor on eosinophils is involved in defense against parasites. Nature 367:183–186, 1994.
26. Capron A, and Dessaint JP. Immunologic aspects of schistosomiasis. Ann Rev Med 43:209–218, 1992.

27. El Ridi R, Ozaki T, and Kamiya H. *Schistosoma mansoni* infection in IgE-producing and IgE-deficient mice. J Parasitol 84:171–174, 1998.
28. Sher A, Coffman RL, Hieny S, et al. Ablation of eosinophil and IgE responses with anti-IL-5 or anti-IL-4 antibodies fails to affect immunity against *Schistosoma mansoni* in the mouse. J Immunol 145:3911–3916, 1990.
29. Shioda H, and Mishima K. Significance of mast cells in nasal smears from patients with allergic disorders. J Asthma Res 3:323–324, 1966.
30. Metzger H. The receptor on mast cells and related cells with high affinity for IgE. Contemp Top Mol Immunol 9:115–145, 1983.
31. Fischer B, and Schmutzler W. Histamine release from isolated mast cells as a bioassay system for the standardization of allergens. Agents Actions 11:103–105, 1981.
32. Burrows B, Martinez FD, Halonen M, et al. Association of asthma with serum IgE levels and skin-test reactivity to allergens. N Engl J Med 320:271–277, 1989.
33. Freidhoff LR, and Marsh DG. Relationship among asthma, serum IgE levels, and skin test sensitivity to inhaled allergens. Int Arch Allergy Immunol 100:355–361, 1993.
34. Kinet JP, Blank U, Brini A, et al. The high-affinity receptor for immunoglobulin E: A target for therapy of allergic diseases. Int Arch Allergy Appl Immunol 94:51–55, 1991.
35. McDonnell JM, Beavil AJ, Mackay GA, et al. Structure based design and characterization of peptides that inhibit IgE binding to its high-affinity receptor. Nat Struct Biol 3:419–426, 1996.
36. Chang TW, Kung PC, Gingras SP, et al. Does OKT3 monoclonal antibody react with an antigen-recognition structure on human T cells? Proc Natl Acad Sci USA 78:1805–1808, 1981.
37. Russell PS, Colvin RB, and Cosimi AB. Monoclonal antibodies for the diagnosis and treatment of transplant rejection. Annu Rev Med 35:63–81, 1984.
38. Chatenoud L, Baudrihaye MF, Chkoff N, et al. Restriction of the human in vivo immune response against the mouse monoclonal antibody OKT3. J Immunol 137:830–838, 1986.
39. Nakagawa T, Stadler BM, Heiner DC, et al. Flow-cytometric analysis of human basophil degranulation. II. Degranulation induced by anti-IgE, anti-IgG4 and the calcium ionophore A23187. Clin Allergy 11:21–30, 1981.
40. Kimura I, Tanizaki U, Sato S, et al. Differences in response to anti-IgE and to anti-IgG in basophils from patients with bronchial asthma. Clin Allergy 11:31–36, 1981.
41. Hook WA, Zinsser FU, Berenstein EH, et al. Monoclonal antibodies defining epitopes on human IgE. Mol Immunol 28:631–639, 1991.
42. Haba S, and Nisonoff A. Effects of syngeneic anti-IgE antibodies on the development of IgE memory and on the secondary IgE response. J Immunol 152:51–57, 1994.
43. Stadler BM, Gang Q, Vogel M, et al. IgG anti-IgE autoantibodies in immunoregulation. Int Arch Allergy Appl Immunol 94:83–86, 1991.
44. Kolbinger F, Saldanha J, Hardman N, et al. Humanization of a mouse anti-human IgE antibody: A potential therapeutic for IgE-mediated allergies. Protein Eng 6:971–980, 1993.
45. Casale TB, Bernstein IL, Busse WW, et al. Use of an anti-IgE humanized monoclonal antibody in ragweed-induced allergic rhinitis. J Allergy Clin Immunol 100:110–121, 1997.
46. Liu J, Lester P, Builder S, et al. Characterization of complex formation by humanized anti-IgE monoclonal antibody and monoclonal human IgE. Biochemistry 34:10474–10482, 1995.
47. MacGlashan DW Jr, Bochner BS, Adelman DC, et al. Down-regulation of FcϵRI expression on human basophils during in vivo treatment of atopic patients with anti-IgE antibody. J Immunol 158:1438–1445, 1997.
48. Saini SS, MacGlashan DW Jr, Sterbinsky SA, et al. Down-regulation of human basophil IgE and FC epsilon RI alpha surface densities and mediator release by anti-IgE-infusions is reversible in vitro and in vivo. J Immunol 162:5624–5630, 1999.
49. Beck LA, Marcotte GV, MacGlashan D, et al. Omalizumab-induced reductions in mast cell Fce psilon RI expression and function. J Allergy Clin Immunol 114:527–530, 2004.
50. Prussin C, Griffith DT, Boesel KM, et al. Omalizumab treatment downregulates dendritic cell FcϵRI expression. J Allergy Clin Immunol 112:1147–1154, 2003.
51. Muddle GC, Bheekha R, and Bruijnzeel-Koomen CA. Consequences of IgE/CD23-mediated antigen presentation in allergy. Immunol Today 16:380–383, 1995.
52. Kuehr J, Brauburger J, Zielen S, et al. Efficacy of combination treatment with anti-IgE plus specific immunotherapy in polysensitized children and adolescents with seasonal allergic rhinitis. J Allergy Clin Immunol 109:274–280, 2002.
53. Rolinck-Werninghaus C, Hamelmann E, Keil T, et al. The coseasonal application of anti-IgE after preseasonal specific immunotherapy decreases ocular and nasal symptom scores and rescue medication use in grass pollen allergic children. Allergy 59:973–979, 2004.
54. Hamelmann E, Rolinck-Werninghaus C, and Wahn U. Is there a role for anti-IgE in combination with specific allergen immunotherapy? Curr Opin Allergy Clin Immunol 3:501–510, 2003.
55. Davis FM, Gossett LA, and Chang TW. An epitope on membrane-bound but not secreted IgE: Implications in isotype-specific regulation. Biotechnology (NY) 9:53–56, 1991.
56. Holgate S, Casale T, Wenzel S, et al. The anti-inflammatory effects of omalizumab confirm the central role of IgE in allergic inflammation. J Allergy Clin Immunol 115:459–465, 2005.
57. Bousquet J, Wenzel S, Holgate S, et al. Predicting response to omalizumab, an anti-IgE antibody, in patients with allergic asthma. Chest 125:1378–1386, 2004.
58. Vignola AM, Humbert M, Bousquet J, et al. Efficacy and tolerability of anti-immunoglobulin E therapy with omalizumab in patients with concomitant allergic asthma and persistent allergic rhinitis: SOLAR. Allergy 59:709–717, 2004.
59. Fahy JV, Fleming HE, Wong HH, et al. The effect of an anti-IgE monoclonal antibody on the early- and late-phase responses to allergen inhalation in asthmatic patients. Am J Respir Crit Care Med 155:1824–1834, 1997.
60. Sampson HA. Update on food allergy. J Allergy Clin Immunol 113:805–819, 2004. □

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