

Anti-IgE Antibodies for the Treatment of IgE-Mediated Allergic Diseases

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Abstract

The pharmacological purposes of the anti-IgE therapy are to neutralize IgE and to inhibit its production to attenuate type I hypersensitivity reactions. The therapy is based on humanized IgG1 antibodies that bind to free IgE and to membrane-bound IgE on B cells, but not to IgE bound by the high-affinity IgE.Fc receptors on basophils and mast cells or by the low-affinity IgE.Fc receptors on B cells. After nearly 20 years since inception, therapeutic anti-IgE antibodies (anti-IgE) have been studied in about 30 Phase II and III clinical trials in many allergy indications, and a lead antibody, omalizumab, has been approved for treating patients (12 years and older) with moderate-to-severe allergic asthma. Anti-IgE has confirmed the roles of IgE in the pathogenesis of asthma and helped define the concept "allergic asthma" in clinical practice. It has been shown to be safe and efficacious in treating pediatric allergic asthma and treating allergic rhinitis and is being investigated for treating peanut allergy, atopic dermatitis, latex allergy, and others. It has potential for use to combine with specific and rush immunotherapy for increased safety and efficacy. Anti-IgE thus appears to provide a prophylactic and therapeutic option for moderate to severe cases of many allergic diseases and

conditions in which IgE plays a significant role. This chapter reviews the evolution of the anti-IgE concept and the clinical studies of anti-IgE on various disease indications, and presents a comprehensive analysis on the multiple intricate immunoregulatory pharmacological effects of anti-IgE. Finally, it reviews other approaches that target IgE or IgE-expressing B cells.

1. Introduction

1.1. The Current Status of the Anti-IgE Development

A therapeutic anti-IgE antibody is a monoclonal antibody (MAb) designed to target IgE and IgE-expressing B cells without the complication of its cross-linking IgE bound by the high-affinity IgE.Fc receptors (also called type I IgE.Fc receptors, FcεRI) on basophils and mast cells. Such an antibody is distinctively different from a common anti-IgE antibody, which can bind to and cross-link IgE-FcεRI and hence sensitize the effector cells bearing them to discharge various pharmacological mediators. In this chapter, a therapeutic anti-IgE antibody designed above is referred to as “anti-IgE,” as has been used routinely in the allergy and asthma fields.

Historically, research on IgE has arrived at an interesting juncture. IgE was discovered in 1967 by Johansson (Johansson, 1967) and Ishizakas (Ishizaka and Ishizaka, 1967), and 20 years later in early 1987, the anti-IgE concept was invented by one of the authors (Chang) of this chapter. In 2007, it will be 20 years since the anti-IgE concept was proposed and research on developing the first antibody prototype initiated (Chang *et al.*, 1990). Today, various immunoassays relating to IgE are now essential tools in the care of allergic diseases, and the initial application of the anti-IgE therapy for treating moderate-to-severe allergic asthma has been approved by health agencies in many countries—it has been used to treat more than 60,000 patients with difficult-to-treat allergic asthma.

The diagram in Fig. 1 summarizes the main events in developing the anti-IgE program. Initially, CGP51901 (a chimeric anti-IgE derived from mouse MAb TESC-21; Davis *et al.*, 1993), which was studied in a Phase I and II clinical trial (Corne *et al.*, 1997; Racine-Poon *et al.*, 1997), and CGP56901 (or TNX-901, a humanized anti-IgE based on TESC-21; Kolbinger *et al.*, 1993) were developed in one corporate program. Later, omalizumab (a humanized anti-IgE, also referred to as E25) emerged in another corporate program (Presta *et al.*, 1993). In 1996, the two programs were combined and omalizumab was chosen for further development on the basis of its superior manufacturing process. TNX-901, which was shown to be safe and efficacious in a Phase II trial on allergic rhinitis (using CGP51901) and in a Phase II trial on peanut allergy (Leung *et al.*, 2003), should serve as a backup drug candidate.

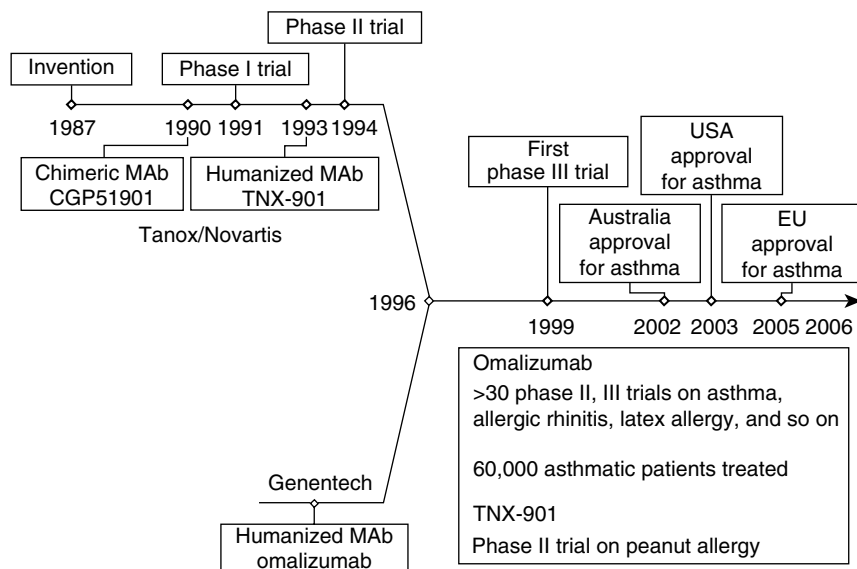


Figure 1 The major events in the development of the anti-IgE therapeutic approach.

Among the key events in the clinical development of the anti-IgE concept are that omalizumab was approved by the United States in 2003 and by the European Union in 2005 for use in treating patients with moderate-to-severe allergic asthma. Omalizumab has now been studied in nearly 30 Phase II and III human trials in various allergic diseases and conditions (Sections 3–5).

1.2. The Main Chemical Features of the Anti-IgE Therapeutic

For most clinicians treating allergic diseases, omalizumab would appear as a very different drug among the battery of drugs for treating allergic diseases. An anti-IgE is a protein, a macromolecular ($\sim 150,000$ Da) biological substance; it is a humanized IgG ($\gamma 1, \kappa$) antibody or a recombinant antibody, which has been substantially improved by genetic engineering methodologies for *in vivo* use in human patients. In overall structure, chemical and physical properties (such as kinetic properties), and ability to mediate a wide spectrum of Fc-related immune mechanisms, a recombinant, humanized anti-IgE IgG1 is similar to an authentic human IgG1. Only the three short complementarity-determining regions (CDRs) in the V_H domain and the three CDRs in the V_κ domain from the parental mouse antibody are retained; nearly the entire four framework segments in each of the V_H and the V_κ are derived from sequence-matched human V_H and V_κ ; the entire CH1, CH2, and CH3 domains of the heavy chain are from human $\gamma 1$ and the C_κ

domain of the light chain is from human κ (Kolbinger *et al.*, 1993; Presta *et al.*, 1993). A striking feature of omalizumab and TNX-901 is that, like a human IgG1, they circulate in the treated patients with a half-life of about 21 days.

Omalizumab is presently provided by the manufacturers in a dry powder formulation, which requires the reconstitution with water to resume a solution form for subcutaneous injection (Strunk and Bloomberg, 2006). Other formulations such as a solution in prefilled syringes for easier administration are under development. Unlike other small molecular compounds synthesized in organic chemical plants, omalizumab is produced by a host Chinese hamster ovary (CHO) cell line in 12,000- or 15,000-liter bioreactor tanks. CHO cell line has become a standard for producing protein pharmaceuticals for human applications (Wurm, 2004). In our case, the CHO cell line was engineered by transfecting with the recombinant “humanized” genes coding the improved $\gamma 1$ and κ chains. The CHO cells express the exogenously introduced antibody genes and produce the humanized antibody in very high yields.

1.3. IgE-Mediated Allergy Is a Vast Medical Field

IgE is well known for its roles in mediating type I hypersensitivity reaction (Gould *et al.*, 2003; Janeway *et al.*, 2005). Through the work of many researchers in the last few decades and the clinical studies of anti-IgE more recently, IgE is now known to play important roles in many allergic diseases. Allergic diseases are generally defined as significant pathological changes that are caused by excessive reactions of the immune system to innocuous substances which the patients are exposed to. While allergic reactions to some substances involves nearly entirely type II, III, or IV hypersensitivity reactions (Janeway *et al.*, 2005), most allergic reactions to inhaled or ingested protein substances involves at least partly type I hypersensitivity reaction and IgE (Oettgen and Geha, 1999). The main allergic diseases or conditions that involve IgE by some extent include allergic asthma (Menz *et al.*, 1998; Oettgen and Geha, 2001), allergic rhinitis (Bodtger *et al.*, 2006; Tschopp *et al.*, 1998) and conjunctivitis (Mimura *et al.*, 2004), allergic or anaphylactic reactions to certain foods (such as peanuts, tree nuts, shell fish, and so on; Sabra *et al.*, 2003), allergic reactions to certain drugs (such as protamin and heparin; Sicherer and Leung, 2005; Weiss *et al.*, 1989), allergic reactions to insect bites (especially wasp and fire ant stings; King and Spangfort, 2000; Schafer and Przybilla, 1996), atopic dermatitis (Leung, 1993), allergic reactions to natural rubber latex (Ebo and Stevens, 2002), allergic reactions to certain raw materials (such as papain, subtilisin, yeasts, and so on) (Baur, 1979; Lemiere *et al.*, 1996) or products in factories (Bernstein, 1997), and allergic reactions to other less common harmless substances.

Many of the allergic diseases mentioned above affect ever-increasing population in most regions of the world (Isolauro *et al.*, 2004). The prevalence is related to economical development (Section 7.1) (Gold and Wright, 2005) and in developed countries the aggregate rates of cases of allergic diseases that are serious enough to seek doctors' help are more than 10% generally and maybe 20–30% in some regions. These diseases affect the health and the quality of life (sleep, school, work, family, and so on) of millions of patients and consume large amounts of healthcare resources.

1.4. The Scope of This Chapter

An earlier review (Chang, 2000) by the lead author of this chapter presented an overview of the rationale and pharmacological basis of the anti-IgE therapy. Since then, a lot of progress relating to anti-IgE has been made. A large number of review articles have been published on anti-IgE, especially reviews summarizing the clinical trials on allergic asthma and discussing the utility of this treatment modality in managing asthma (Busse, 2001; Holgate *et al.*, 2005a; Lanier *et al.*, 2003; Milgrom, 2004; Strunk and Bloomberg, 2006). In this chapter, we will focus on aspects of the anti-IgE concept and development, which have been largely left out by most previous anti-IgE reviews. We will discuss the rationale behind the anti-IgE invention, the clinical development of anti-IgE on various disease indications, and present a comprehensive analysis on the multiple intricate immunoregulatory pharmacological effects of anti-IgE. We will also discuss the development of other approaches that target IgE and IgE-expressing B cells.

2. Rationale Leading to the Invention of the Anti-IgE Concept

2.1. IgE Isotype-Specific Control and IgE Targeting

The idea of isotype-specific suppression of antibodies had already been pursued academically (Bich-Thuy *et al.*, 1984; Hoover and Lynch, 1983) by researchers before the anti-IgE approach was conceptualized. In the field of IgE suppression, a group led by Haba and Nisonoff investigated the potential of inducing intolerance to IgE in mice by administering IgE within a few days after birth when the mice did not produce any IgE. This could suppress the production of IgE even when the mice grew to adulthood. After the anti-IgE concept became known, Haba's group identified syngeneic anti-IgE antibodies in the mice that had been injected with IgE at a neonatal age. They also found that IgE-secreting B cells in these mice were substantially reduced (Haba and Nisonoff, 1990, 1994).

Among the antibody isotypes, substantial suppression would not seem logical except perhaps for IgE. While there is a general belief that IgE plays a role

in the defense against parasitic worms, especially helminthes (Capron and Capron, 1994), the results from many studies could best be grossly characterized as suggestive, but not conclusive (Pritchard, 1993; to be discussed in more detail in Section 13.1). Up until 1980s and even until now, most of the research done on IgE since its discovery in 1967 had been related to the adverse effects of IgE on allergic diseases. The activity of mast cells and basophils in inflammatory processes associated with allergenic responses (Lewis and Austen, 1981; Wasserman, 1989) and the roles of IgE in sensitizing those inflammatory cells had become established (Metzger *et al.*, 1982; Razin *et al.*, 1983). Thus, therapeutic approaches that could decrease the production of IgE would seem to be very logical.

The concentrations of IgE in most patients with allergy are minute, ranging from less than 30–1000 international units (IU) per milliliter (1 IU = 2.4 ng), which corresponds to a total amount of 12 mg or less in the entire body (calculated based on a total volume of 5 liters of body fluid in a person), for more than 90% of the patients (Manz *et al.*, 2005). The B cells, which express mIgE as part of the B cell receptor and can potentially differentiate to IgE-producing plasma cells, also account for minute proportions among lymphocytes (Kasaian *et al.*, 1995). Thus, it appears that IgE and mIgE-expressing B cells are very attractive therapeutic targets.

A few years before setting foot on the anti-IgE program, the lead author (Chang) of this chapter, had been carrying out research on the OKT3 antibody, a mouse MAb against a human T cell surface component, in another corporation. Chang *et al.* (1981) proposed that the antigen recognized by OKT3 is part of then yet unidentified T cell receptor linking to a signal-transducing process. In the next few years, the α/β and δ/γ chains of the T cell receptors were discovered and OKT3 antigen, which was later renamed as CD3 in an International Leukocyte Association, was found to be indeed part of the T cell receptor. While Chang's interest on OKT3 was mainly academic, to his amazement, the antibody was developed by the corporation as an immunosuppressive agent and approved by the US Food and Drug Administration (FDA) in 1986 for use in preventing organ rejection in kidney transplantation (Weimar *et al.*, 1988). OKT3 is the first approved therapeutic antibody. It acts on the T cell receptor and causes T cell depletion. In the spring allergy season in 1987, Chang, being affected by severe allergic rhinitis himself, drew from the experience on OKT3 and designed the anti-IgE approach.

2.2. The Unique Set of Anti-IgE-Binding Specificities

On the basis of the rationale in the Section 2.1, free IgE in the blood and in interstitial fluid and mIgE-expressing B cells, which include mIgE-committed B lymphoblasts and memory B cells, are very attractive therapeutic targets by

specific immunological agents. On the surface of mIgE-expressing B cells, a specific cell surface marker that is unique for those B cells and that is reachable by an immunological agent had not been identified, except perhaps mIgE itself. mIgE is part of the B cell receptor, which is linked via associated subunits to signal-transducing pathways (DeFranco, 1993; Niiro and Clark, 2002), on B cells expressing mIgE. This makes mIgE a viable immunological site to target for modulating mIgE-expressing B cells. Thus, an antibody could potentially be designed to target free IgE in the body fluid and mIgE on B cells simultaneously, because the relatively small amounts of soluble, free IgE should not consume or neutralize a potential targeting antibody prohibitively.

The trick in designing an antibody to target IgE and mIgE was that the antibody must not bind to IgE that is already bound by FcεRI. It had been known that antibodies made against IgE could sensitize basophils and mast cells and, in fact, several laboratories had used these antibodies to study the mechanisms of activation of those effector cells (Conroy *et al.*, 1977; Ishizaka *et al.*, 1984). By applying hybridoma methodology and a series of screening procedures, hybridoma clones secreting MAbs with the set of desired specificities were identified. Figure 2 summarizes the binding specificities of anti-IgE found at the time of its discovery and in later studies.

The selected MAbs have high binding affinity to human IgE (K_d about 1×10^{-10} M), for they are designed to compete with the high-affinity IgE receptor, FcεRI, for binding to IgE (Corne *et al.*, 1997; Davis *et al.*, 1993). The MAbs have no crossbinding activities toward other serum substances and other cell types. They were examined for crossbinding activities to various histological tissue sections, which represented all tissues in the human body, and found to be free of such activities.

2.3. Structural Basis of the Unique Binding Specificities

The mouse antihuman IgE MAbs with the required set of binding specificities were obtained before the 3D structures of IgE, its high-affinity receptor, FcεRI, and low-affinity receptor, FcεRII, had been solved. The bent conformation of IgE and of the 1:1 binding stoichiometry between the α chain of FcεRI and IgE were elucidated by various approaches (Keown *et al.*, 1997; Zheng *et al.*, 1991). The X-ray structures of the FcεRI α chain, free (PDB entry 1f2q, 2.4 Å; Garman *et al.*, 1998) and complexed with human IgE CH3–CH4 domains (PDB entry 1f6a, 3.5 Å; Garman *et al.*, 2000), have provided a structural basis for understanding the binding properties of the anti-IgE MAbs (see below). The structure of FcεRII, also known as CD23, and the characteristics of its binding to IgE have also been elucidated in detail (Hibbert *et al.*, 2005; Wurzburg *et al.*, 2006).

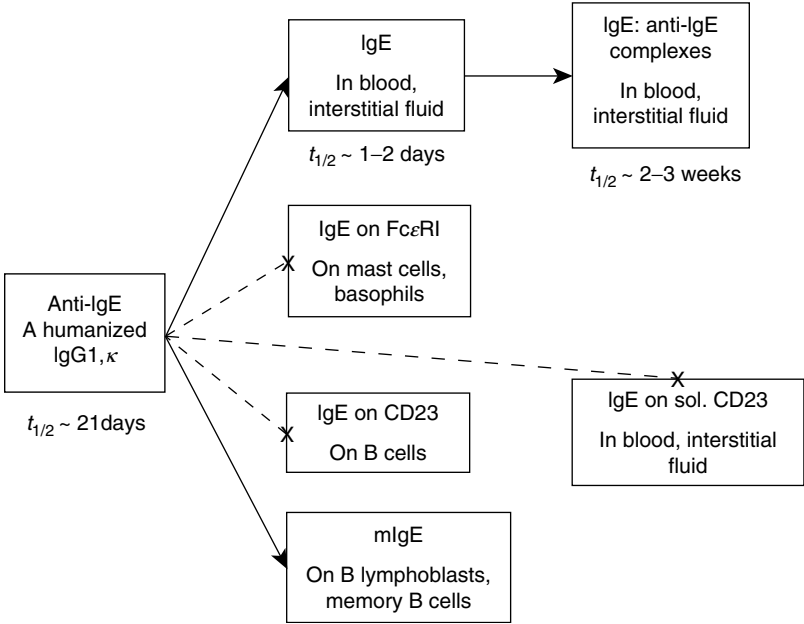


Figure 2 The unique set of binding specificities an anti-IgE antibody. The half-lives of IgE, anti-IgE, and the immune-complexes of IgE and anti-IgE are also indicated.

It is now clear that both omalizumab and TNX-901 IgG1 antibodies, the FcεRI α chain, and CD23 bind to the CH3 domain of IgE. They affect the binding of each other, suggesting that their binding sites on IgE either overlap or are probably in proximity to one another. The binding of IgE by anti-IgE will prevent IgE from binding to FcεRI and to CD23 on B cells. Conversely, the binding of IgE by FcεRI will prevent anti-IgE from binding to IgE. The binding of IgE by CD23 will also block anti-IgE binding to IgE. At first glance, such an explanation seems straight forward, based on a simple understanding that the binding of IgE by one molecule will sterically hinder the access by another. In other words, the binding of IgE by one entity masks the binding site for another. This would be perfectly rationale, had there been only one CH3 domain in one IgE molecule. However, there are two CH3 domains (two ε chains) in one IgE molecule.

The Fc (CH2–CH3–CH4 region) of IgE exists in a bent conformation, with the CH2 lobes bending toward one side by a large angle against the two CH3 lobes and hanging not distant from the CH4 lobes (Wan *et al.*, 2002). The two trunks of CH2–CH3–CH4 domains do not have an axis of twofold symmetry between them. The two CH3 domains have a pseudo dyad axis between them;

the two CH4 domains also have one; the two dyad axes are offset by 3° . The two CH2 domains are linked to their respective CH3 domains by linker segments that are part of CH3 and lay crossed. Looking down from atop, the two CH2 domains and two CH3 domains are like a bent letter “X.”

The fact that one IgE molecule can be bound by two anti-IgE molecules may explain why anti-IgE blocks IgE binding to CD23 or FcεRI. The membrane-bound form of CD23 exists on the cell surface as a trimer, each of which extends a C-type lectin-like “head” domain through a long “stalk” and a “neck” structure (Hibbert *et al.*, 2005). The head domain binds to the outer side of a CH3 domain of IgE in a Ca^{2+} -dependent, nonlectin-like manner (independent of carbohydrates). A single head domain of CD23 binds to IgE with very low affinity ($K_d = 10^{-5}$ M) and hence two of the heads must bind to two CH3 domains to hold the IgE in place ($K_d = 10^{-8}$ M). Thus, the binding by CD23 precludes anti-IgE to bind to IgE simultaneously.

Figure 3 shows that the two CH3 domains and two CH4 domains form a rhombic shape with a large opening in the center. The α chain of FcεRI binds to both CH3 domains (indicated by shaded parts), which is uncommon in receptor–ligand interaction. The wide opening of the two CH3 lobes allows the α chain to bind to the inner sides of the CH3 domains. Even more strikingly, the two sites on the CH3 domains bound by the α chain of FcεRI, with one larger than the other, share 4-amino acid residues between their respective CH3 peptide chains. The binding by α chain of FcεRI causes significant conformational change pushing apart the two CH3 domains and enlarging the opening between them (compare Fig. 3A and C, also D and E).

It is not yet solved where on the dimeric CH3 domains the two anti-IgE molecules bind to. Presumably, they are near the binding site for the α chain of FcεRI such that binding by the α chain of FcεRI would hinder both anti-IgE molecules to bind to. However, the fact that CH2 domains bend to and hang over closely one side of the CH3 domains would suggest that the binding sites for anti-IgE should not be near the inner sides of the CH3 lobes (because two anti-IgE molecules can bind to one IgE molecule). A crystal structure of CH3 and the Fv of anti-IgE would be of great interest.

2.4. Prevailing Concepts at the Time of the Invention

At the present time, the prospects of anti-IgE as a treatment option for many allergic diseases look promising. However, leaders in the field of allergy and immunology were largely skeptical about the approach in the early years of the program. In the realm of pharmaceutical development in the late 1980s, the thrust was to identify, by a combination of compound screening and organic synthesis, small molecules (as opposed to protein drugs) that could block

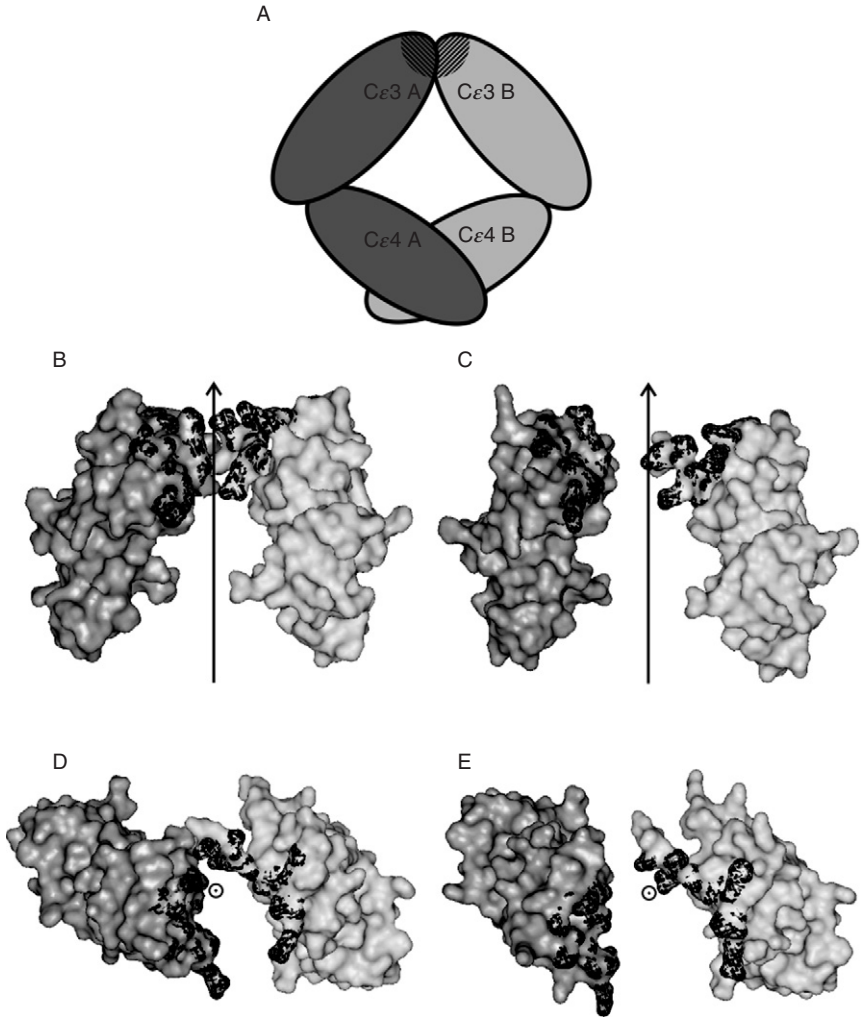


Figure 3 The binding site of the α chain of Fc ϵ RI on CH3 domains. (A) The two CH3 lobes and two CH4 lobes form a rhombic shape with an opening in the center; the binding site for α chain is on the inner sides of both CH3 lobes, near the junctions with CH2 lobes. (B) The “frontal” view of CH3 domains before α chain binding. (C) The frontal view of CH3 domains after α chain binding. (D) The “top” view of CH3 domains before α chain binding. (E) The top view of CH3 domains after α chain binding. The structures show that the binding by α chain causes the opening of the two CH3 lobes wider. The binding sites for Fc ϵ R II (CD23) and potential binding sites for anti-IgE are discussed in the text.

targeted biological processes. In the field of allergy, most of the pharmaceutical research was to develop drugs that could be used to block leukotrienes, tryptase, or other inflammatory mediators (Barnes, 2004). Researchers had also started to screen compound libraries for drug leads that can bind to the α chain of Fc ϵ RI and block its binding by IgE. The notion of using an antibody to treat allergic disease was beyond the imagination of most academic researchers and clinicians then (Chang, 2006).

An immediate feeling among scientists first told of the anti-IgE approach was that other anti-IgE antibodies had already been known to be potent inducers of basophil and mast cell activation, and would cause anaphylactic reactions, if they were injected into a person. Hence, the idea of injecting an anti-IgE antibody, albeit different from ordinary anti-IgE antibodies, was met with skepticism. Among other major arguments put forth by immunologists was that even if IgE could be neutralized to better than 99%, the remainder would still be sufficient to charge the receptors on basophils and mast cells, keeping them sensitive to allergens (Pruzansky and Patterson, 1988). Another thought was that as IgE is bound by the high-affinity receptors on mast cells for many months or perhaps more than a year, the neutralization of free IgE by administering anti-IgE would not affect the sensitivity of those cells. Yet another argument was that IgE is essential for immune defense functions and cannot be compromised (Capron and Capron, 1994; Capron and Dessaint, 1992). Despite these overall dubious sentiments, the new therapeutic concept eventually found a few supporters from a large pharmaceutical company and has been actively developed, although bumps were hit on the way, in the last 20 years. Officials and scientists at the FDA in the United States and at the Medicines Control Agency (now reorganized) in United Kingdom encouraged the development of this drastically different experimental drug for allergic diseases with cautious interest and provided guidance to us in preparing for the first human experimentation. The chimeric anti-IgE antibody, CGP51901, was first administered to patients in Southampton, United Kingdom in 1991. Initially, while we had difficulty dissecting the biological results, mainly the rapidly rising total IgE (accumulating immune complexes), safety was not a major concern.

3. Anti-IgE Is Approved for Treating Moderate-to-Severe Asthma

In 1994–1995, the first Phase II trial of anti-IgE was performed with CGP51901 at three medical centers in Central Texas on patients with severe allergic rhinitis caused by mountain cedar pollens. The patients were randomized in four groups and given weekly injections of placebo or CGP51901 at

15-, 30-, or 60-mg dose. The results revealed a robustly clear dose response, showing that the antibody was safe and efficacious in alleviating nasal and ocular allergic symptoms (results not published; the lead author of this chapter participated in the study).

In 1996, the two corporate anti-IgE development programs were combined (Fig. 1). The new partnership planned clinical development paths for omalizumab and decided that several Phase II trials be carried out on both allergic asthma and allergic rhinitis. As the development program proceeded in the following years, increased emphasis was placed on allergic asthma. It was reasoned that the incidences of asthma, especially pediatric asthma, were rising in most regions of the world at alarming rates (Cookson, 1999; Kussin and Fulkerson, 1995). Also, the most severe cases (10%) of asthma are life-threatening and consume large amounts of healthcare resources. In the new awareness of pharmacoeconomic environment, the costs of a drug per patient per year must be weighed against the therapeutic benefits achieved and the healthcare dollar saved (Hoskins *et al.*, 2000).

Many excellent review articles have been written by clinical investigators summarizing and analyzing in great detail the results obtained in the series of trials of omalizumab on allergic rhinitis. To avoid repeating such a task, this section will summarize the clinical studies succinctly for immunologists, who normally do not survey clinically oriented journals, and discuss aspects of the effects of anti-IgE on asthma that are not covered by other reviews.

3.1. "Allergic Asthma" Has Been Adopted as a New Clinical Indication

"Allergic asthma," a term that had already appeared in the literature in the 1950s (Prickman, 1951) but used sparingly in the ensuing few decades, has emerged as an important concept as the anti-IgE concept gradually takes hold. Practically, most clinicians treating asthma patients do not need to know possible immunological or allergic involvement in the asthmatic disease they treat. They use corticosteroids to suppress inflammatory responses and β_2 adrenergic receptor agonists to relax constricted airway smooth muscles.

In pharmaceutical development, a candidate drug is studied for use to treat a defined clinical indication. Normally, a drug being developed for asthma, such as a new chemical entity or a new formulation of β_2 agonist, antileukotriene, or corticosteroid, is tested for treating asthma without considering the asthmatic cases' possible allergic nature. The frequent news and official reports of the clinical study results of the anti-IgE drug candidates in the past decade have gradually brought an awakening among clinicians treating asthma patients that in a large proportion of asthma cases, allergy is involved (Holgate *et al.*, 2005a). In the clinical trials of anti-IgE, a skin prick test was required to

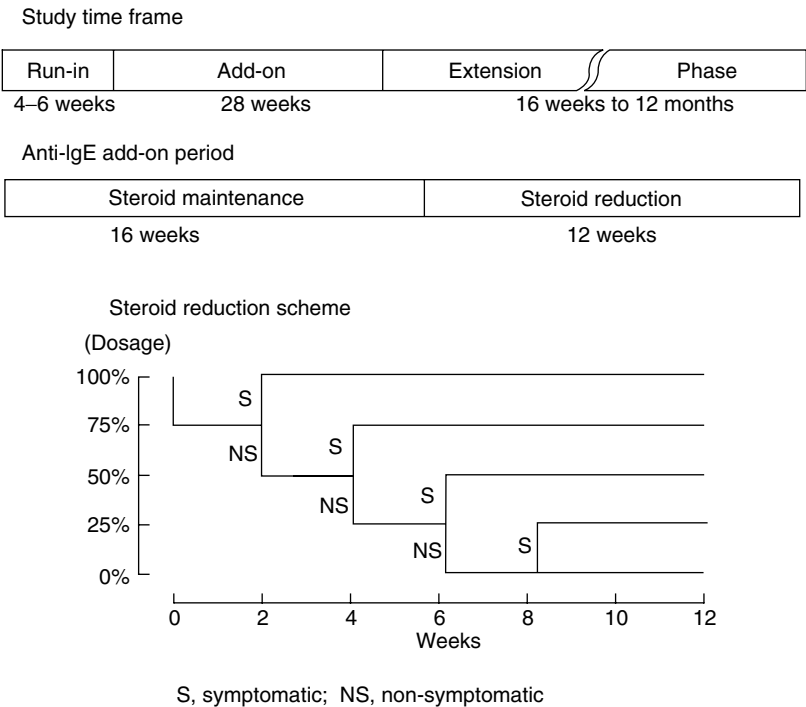
screen patients for positive reactivity toward at least one allergen suspected to be the cause of potential allergy in the examined patient. Now that omalizumab has been approved for marketing for “allergic asthma,” clinicians use positive skin prick test reactivity as one criterion to determine the suitability of an asthma patient to receive omalizumab medication.

3.2. Clinical Parameters Determined in the Clinical Studies

The clinical studies of omalizumab for treating patients with allergic asthma, with either moderate-to-severe or severe disease, in either adolescent and adult or pediatric populations, with or without the combination of other drugs, all adopted a general protocol design (Fig. 4; Buhl *et al.*, 2002; Milgrom *et al.*, 2001; Soler *et al.*, 2001), which has been regarded as highly effective. As a major clinical objective of the new anti-IgE drug was to reduce corticosteroid use, a key component in the clinical study design was that it enabled the examination whether the patients’ dependence on corticosteroids can be partially reduced or withdrawn entirely.

In the multicenter, double-blinded clinical studies, patients with allergic asthma were recruited with a set of screening criteria and randomized into placebo and treatment groups. Generally, patients were allowed a 4- to 6-week “run-in” period to establish their use of adequate amounts of corticosteroids, then a 28-week core-study or “add-on” period, in which the patients were given placebo or omalizumab of 150- or 300-mg dose every 2 or 4 weeks according to their plasma IgE levels and body weights, and lastly, a 4-, 6-, or 12-month “extension period” for continual observation (Fig. 4). In the first 16 weeks of the 28-week add-on period, patients were maintained at their regular doses of corticosteroid; in the last 12 weeks of the 28-week add-on period, a “steroid reduction” schedule was implemented for each patient. Beginning at the starting point of the 12-week period, a quarter of the stabilized amount of corticosteroid was taken off. If, in the 2-week interval, the patient maintained symptom-free, another quarter amount of the steroid would be reduced, whereas if a patient became symptomatic as defined by the chart in Fig. 4, the steroid would be resumed to the dose of 2 weeks before and kept at that dose throughout the remaining period of the trial (Buhl *et al.*, 2002; Milgrom *et al.*, 2001; Soler *et al.*, 2001).

To date, a total of 17 clinical studies of omalizumab on allergic asthma, covering adult, adolescent, and pediatric patients, with varying degrees of disease severity, and with different accompanying medications, have been performed and published. Those results clearly indicate that anti-IgE was effective in reducing the numbers of asthma attacks (exacerbations) at the same time when corticosteroids were substantially reduced or completely



Symptomatic criteria (as appeared in Holgate *et al.*, 2004):

1. >50% increase in 24-h rescue medication use on at least 2 of any 3 consecutive days compared to mean use over the last 7 days of the preceding phase.
2. Mean daily asthma symptom score ≥ 4 over the previous 7 days.
3. Fall in morning peak expiratory flow (PEF) of >20% on at least 2 of any 3 consecutive days relative to the mean morning PEF over the last 7 days of the proceeding phase.
4. Worsening of disease between visits requiring an unscheduled practitioner or hospital visit.
5. At least 2 of 3 consecutive nights with awakenings due to asthma symptoms requiring rescue medication.
6. An asthma exacerbation.

Figure 4 The clinical trial protocol used in most of the trials of omalizumab in allergic asthma.

withdrawn in most of the patients (Bousquet *et al.*, 2005; Soler *et al.*, 2001). The numbers of unscheduled doctor’s office visits, emergency department visits, days of hospitalization were also significantly reduced (Bousquet *et al.*, 2005; Humbert *et al.*, 2005). Lung functions, in terms of forced expiratory

volume, were increased (Ayres *et al.*, 2004). The other measurements, which were of concern to the patients and the clinicians and whose changes were statistically significant, included many quality-of-life measurements, including reduction of missing days to school or to work and loss of sleep (Ayres *et al.*, 2004; Buhl *et al.*, 2002; Lemanske *et al.*, 2002).

3.3. The Clinical Studies Confirm the Roles of IgE in the Pathogenesis of Asthma

At the time when the anti-IgE therapeutic concept was conceptualized and during its initial clinical development, it was still debated whether IgE is involved in asthma. The situation was different in allergic rhinitis, where there was a general belief that IgE is involved in the disease. In the United States and Western European countries, clinicians treating moderate to severe cases of allergic rhinitis were mostly allergists, who were trained in the specialty of allergy. These clinicians were familiar with allergen-based immunotherapy and applied it to treat patients. They used patch tests and skin prick tests to gauge immune responses to suspected allergens and measured plasma total IgE and allergen-specific IgE levels to assist their therapeutic practice. Immunology and IgE have been a big part of the knowledge and tool base of the medicine applied for allergic rhinitis (Sections 4.1 and 5).

Asthma patients were generally not treated by allergists, but by pulmonary or thoracic physicians, or internists, who did not receive substantial amounts of immunology in their training. These clinicians treated mainly the inflammation and bronchial constriction associated with asthma. The knowledge on possible involvement of the immune system or IgE in asthma would not matter in their care of asthmatic patients. Very few cases of asthma are treated with immunotherapy. Perhaps the most relevant research linking IgE to asthma were a number of surveys investigating the correlation between (1) asthma and serum IgE levels (Burrows *et al.*, 1989) and (2) the severity of asthma and serum IgE levels (Borish *et al.*, 2005). However, the overall clinical setting and the lack of experimental evidence linking directly IgE activities to asthma left open an unsettled dispute whether IgE is involved in the pathogenesis of asthma.

The clinical studies of anti-IgE demonstrate that anti-IgE, by virtue of its ability to incapacitate IgE, can not only inhibit the early-phase reactions, occurring within the first 4 h of an asthma attack, but also the late-phase reactions, occurring beyond the first 4 h (Boulet *et al.*, 1997; Fahy *et al.*, 1997). These inhibitory effects of anti-IgE result in improving various symptoms of allergic asthma, as shown convincingly in more than 15 Phase II and III clinical studies. Thus, anti-IgE has helped to confirm unambiguously the roles of IgE in the pathogenesis of allergic asthma (Holgate *et al.*, 2005a).

3.4. Analyses of Good Responders Among Asthma Patients

For an expensive drug like omalizumab, a diagnostic or screening procedure that helps identify patients who are likely to respond well to and benefit substantially from the drug is highly desirable (Heaney and Robinson, 2005). Asthmatic patients with protracting conditions present variable sets of pathogenic components and probably respond to anti-IgE therapy with different degrees of improvement. In addition to a positive skin test to allergens, criteria such as a suitable range of IgE would be highly desirable for identifying potential good responders.

The various clinical trials of omalizumab on asthma and the corporations marketing omalizumab have indicated that omalizumab achieved response rates in the range of 60–90%. It appeared that when the studied or targeted patients are more severe, the response rates are higher. Statistical analyses performed on the aggregated results of clinical trials on asthma indicate that anti-IgE benefit best the most severe, difficult-to-treat patients, coincidentally matching a pharmacoeconomic criterion (Oba and Salzman, 2004). Most of those difficult-to-treat asthma patients cannot be controlled even with very high doses of inhaled and injectable corticosteroid ($\text{BDP} > 800 \mu\text{g/day}$) and have poor lung functions ($\text{FEV}_1 < 65\%$) (Bousquet *et al.*, 2004). Anti-IgE appears to be able to remove the root cause of inflammation in those difficult-to-treat patients (Ayres *et al.*, 2004; Humbert *et al.*, 2005). Clinicians have also suggested that asthma patients who have concomitant allergic rhinitis also respond well to anti-IgE treatment (Vignola *et al.*, 2004).

The basal levels of IgE do not seem to correlate with responsiveness or degrees of efficacy. In the large number of clinical trials on omalizumab, patients with plasma IgE outside the 30–700 IU range were excluded. The dosing schedule allowed omalizumab to be in excess of replenished IgE during the clinical trial periods and hence IgE levels within this range should make no difference to the effectiveness of anti-IgE. That plasma IgE levels affect the density of $\text{Fc}\epsilon\text{RI}$ on effector cells and the accumulation of anti-IgE:IgE immune complexes will be discussed in Sections 7–9.

4. Studies on Other Allergic Diseases

Among the large populations of patients affected by allergic rhinitis, food allergy (especially peanut allergy), or atopic dermatitis are severe cases, which cannot be adequately treated with currently available drugs and are in need of better medicine. There are smaller patient populations who are affected by sensitivity to occupation-related materials, such as natural rubber latex, papain, yeast, and so on, to certain drugs, or to insect stings. While it is

clear that IgE is involved in the pathogenesis of allergic rhinitis, the information in the literature is not indicative of whether IgE is a dominant factor in other allergic diseases or conditions. Thus, anti-IgE offers not only a valuable tool to study the extent of IgE's involvement in these allergic diseases but also a possible treatment modality for the severe cases of these clinical indications.

4.1. Clinical Applications in Treating Allergic Rhinitis

Ten to forty percent of the general population is affected by allergic rhinitis in different regions of the world (Asher *et al.*, 2006; Schoenwetter, 2000). Proportions of those populations have substantial symptoms and use over-the-counter medicine or seek physicians' help. In the United States, patients visit doctors' offices more times for allergic rhinitis than for any other disease. There are more than 10,000 licensed allergists in the United States (information from American Academy of Allergy, Asthma, and Immunology), who treat mainly patients with allergic rhinitis with immunotherapy.

Unlike most allergic asthmatic cases, which are caused by indoor allergens such as dust mites and cat dander, most allergic rhinitis cases are caused by outdoor airborne protein antigens such as tree pollens in the spring and grass pollens in the late summer and fall (Boulet *et al.*, 1997; Leynaert *et al.*, 1999). This interesting dichotomy is a curious subject for investigation. By mechanisms that are yet not well understood, the exposure of the body to minute amounts of protein antigen at the mucosal surface in the respiratory and the gastrointestinal tracts can induce antigen-specific IgE production in some people. Some patients have sensitivity toward one or very few allergens, while others are sensitive to a large numbers of allergens. Some patients have very defined allergic rhinitis, while others have concomitantly asthmatic and dermatitis symptoms. Some patients have distinctive seasonal patterns, suffering seasonal allergic rhinitis only in the spring when tree pollens are dense, or only in the fall when grass pollens are rampant, or in both seasons, whereas others have perennial allergic rhinitis and are affected by a number of allergens that together are present throughout the whole year (Beck and Leung, 2000; Braunstahl, 2005).

Altogether more than a dozen Phase II and III clinical trials have been performed on allergic rhinitis in the United States, Germany, Scandinavian countries, Australia, New Zealand, Japan, and other countries. These include studies that investigated the efficacy and safety of (1) CGP51901 on patients with severe allergic reactions to mountain cedar pollens and (2) omalizumab on patients with sensitivity on birch pollens, ragweed pollens, pollens of other grasses, and others (Adelroth *et al.*, 2000; Casale, 2001; Casale *et al.*, 1997, 2001; Nayak *et al.*, 2003). In both clinical trials on seasonal allergic rhinitis and

on perennial allergic rhinitis, anti-IgE was shown to be effective in improving allergic rhinitis symptoms, reducing the inflammation and mucous secretions in the nasal and conjunctiva linings and reducing sneezing attacks. The treatment also improved quality of life measurements.

These studies have amply demonstrated that IgE and type I hypersensitivity are clearly a dominant mechanism for most cases of allergic rhinitis. Despite the robust data on the safety and efficacy of anti-IgE in treating allergic rhinitis, governmental regulatory approval of marketing omalizumab for allergic rhinitis indication is not imminent. It appears that FDA requires a sufficiently long postmarketing period to ensure that the long-term use of anti-IgE by asthma patients does not cause undesirable side effects.

4.2. Anti-IgE Studies on Treating Peanut Sensitivity

Researchers working on anti-IgE recognized early the importance of investigating the utility of anti-IgE in treating sensitivity or anaphylactic reactions to peanuts. In economically developed countries, some people, mostly children, develop extreme sensitivity to peanuts (Skolnick *et al.*, 2001). They mount severe allergic reactions, often in the form of anaphylaxis, from ingesting or inhaling peanut product at an amount as small as half a peanut. The incidences are often accidental and life-threatening, hence peanut allergy often causes constant fear in the patients and their families. The treatment of peanut allergy is strict avoidance of peanut-containing food product. Patients and their relatives are advised to carry a prefilled epinephrine (β_2 agonist) syringe for relieving anaphylactic reactions induced by inadvertent exposures. It is also known that allergen-based immunotherapy and corticosteroids do not treat peanut allergy effectively. Thus, a therapy that can substantially reduce patient's sensitivity to peanut would be of great relief for these patients and their families.

TNX-901 was studied in a Phase II clinical trial on peanut allergy (Leung *et al.*, 2003). In the study, 84 volunteering patients were randomized into four groups and given placebo or TNX-901 at 150, 300, or 450 mg subcutaneously every 4 weeks for four times. Two to three weeks after the last injection, the patients were challenged with increasing amounts of encapsulated peanut flour, which the patients swallowed. It was shown that TNX-901 could increase the tolerable amounts with increasing doses of the anti-IgE. At the 450-mg dose, the tolerability was increased from an average of half a peanut to 28 peanuts in most patients. These results do not suggest that the patients can venture out eating peanut packages or peanut-containing food, but the anti-IgE drug will protect patients from anaphylactic reactions, when they accidentally eat peanut-containing meals or food products or breathe in peanut-containing powders.

A Phase II trial of a similar design was also carried out for omalizumab. However, it was terminated in the middle of the trial, because a few patients developed severe hypersensitivity reactions, while they were tested for their baseline levels of sensitivity to peanuts by taking in peanut flour, prior to receiving omalizumab. A new study with a different design will be started later this year (information from news releases from the corporations developing omalizumab). Furthermore, the favorable results of TNX-901 on peanut allergy should encourage clinical studies on allergy toward other foods.

4.3. Clinical Application in Latex Sensitivity

Some people are sensitized to natural rubber latex and products made from it, especially latex gloves for healthcare workers (physicians, dentists, nurses, and so on) and balloons for children (Sussman *et al.*, 2002). Patients are exposed to natural rubber latex by direct skin contact to latex products and by inhaling latex-containing powders used for packing and aiding use of latex products. The most frequent symptoms associated with latex are respiratory and skin complications (Fish, 2002). Most patients develop IgE responses to proteins contained in natural rubber latex. This raises the following intriguing question: if a patient is exposed to latex products mainly by direct skin contact, how do the proteins contained in the latex powder or in the latex sheets induce IgE immune response?

In addition to avoidance of latex products, immunotherapy using natural rubber latex extracts have also been tested on children with latex sensitivity and found to have partial response. A small Phase II trial was performed investigating the efficacy of omalizumab on 18 healthcare workers with allergy to latex products (Leynadier *et al.*, 2004). The patients developed nasal and ocular symptoms and mild-to-moderate asthma on using latex gloves and their sera were shown to contain IgE specific for latex proteins. In the double-blinded study, patients received omalizumab (150–750 mg/month) or placebo for 16 weeks. They were evaluated in terms of “conjunctival challenge test total scores” after 8 and 16 weeks of treatment. The results indicated very convincingly that anti-IgE helps alleviate allergic reactions to natural rubber latex.

4.4. Allergic Diseases or Conditions for Which Anti-IgE Has Been Tested in Case Studies

As the safety and efficacy of anti-IgE have been well established in allergic asthma and allergic rhinitis, clinicians have become adventurous in testing the effects of anti-IgE on diseases that manifest allergic symptoms and are known to be associated with elevated levels of IgE. These tests have not been

performed systematically and generally involve only one or a few patients. Most of these tests have not been published, but a few have been published as case reports. Among many other allergic diseases that are prevalent and have an apparent association with IgE is atopic dermatitis (also called urticaria; Leung and Soter, 2001; Shehade *et al.*, 1988), which affects mainly children at an alarming rate in economically advanced countries (Eigenmann *et al.*, 1998; Novak *et al.*, 2003a). The hallmark of atopic dermatitis is the generally very high blood IgE levels, which are in excess of 1000 IU/ml in most patients and reach more than 10,000 IU/ml in a sizable fraction of patients (Laske *et al.*, 2003; Somos *et al.*, 2001).

The clinical studies of omalizumab only included patients with plasma IgE in the range of 30–700 IU/ml. It was estimated that administering anti-IgE at the designed protocol would maintain anti-IgE in excess of newly synthesized IgE in the treatment period. Thus, the high IgE in atopic dermatitis patients would dissuade use of anti-IgE, which administered at comparable doses could not neutralize the newly synthesized IgE and hence would not be effective. The three reports of off-label use of omalizumab on 3, 3, and 7 patients, respectively, yielded mixed, but promising results. In the first report, the three adult patients aged 34–48, who had baseline serum IgE at 23,000, 5440, and 24,400 IU/ml, respectively, failed to benefit from omalizumab administered at 450-mg dose every other week for 4 months (Krathen and Hsu, 2005). In the second report, three young recalcitrant atopic dermatitis patients 11–13 in age, who had baseline serum IgE in the range of 1990–6120 IU/ml, received omalizumab at 150–450 mg every other week for 22 weeks and experienced substantial improvement. In the third report, seven patients, 7–58 in age, who had persistent asthma and severe atopic dermatitis and baseline serum IgE in the range of 265–2020 IU/ml, received omalizumab at 375 mg every other week for 7 months (two patients got omalizumab for only 3 months because their insurance companies refused to cover the costs of the treatment). All patients improved in their eczema severity scores measured at 3 months (for all seven patients) and 7 months (for five patients) after treatment (Lane *et al.*, 2006; Vigo *et al.*, 2006). It is not clear why the first patient group responded differently from the other two; IgE levels might be a factor (Section 9.2).

Two other very interesting clinical indications have been tested with omalizumab, for which the results have been published. There was a case report on a patient with serious cold-induced urticaria/anaphylaxis (Boyce, 2006). A regimen of 375 mg of omalizumab biweekly resolved the patient's urticaria, asthma, and rhinitis symptoms, with noticeable improvement occurring after the second injection of omalizumab. There was also a case report on a patient with serious interstitial cystitis, which does not seem to be an allergic disease

(Lee *et al.*, 2006). Because the patient also had allergic rhinitis and asthma, specific immunotherapy (SIT) was recommended for her. However, she would develop anaphylactic reactions even to 1000-fold diluted antigens, and hence omalizumab was suggested to tame the anaphylactic reactions. The patient was given omalizumab at 300 mg every 4 weeks. To the surprise of the patient and the treating clinician, the patient's interstitial cystitis was improved on the first injection. While both of those cases cannot be used to support that anti-IgE be approved for these disease indications, they encourage further investigation on the roles of IgE in these diseases and the potential of anti-IgE in treating them. The case reports also provide extraordinary insights into the pharmacological mechanisms of anti-IgE.

5. The Potential of Using Anti-IgE to Assist Allergen-Based Immunotherapy

In economically advanced countries in North America and Western Europe, allergy is a well-defined medical specialty serving substantial medical needs. In this specialty, vast amounts of research and development have been carried out and clinical tools accumulated. Undoubtedly, the most significant body of knowledge base in the allergy specialty is the know-how on immunotherapy. Since immunotherapy was introduced by Noon nearly a century ago (in 1911), a sea of experience has been organized for clinical practice. Clinicians are familiar with the hundreds of allergens present in the local area where they practice. They have established mechanisms for acquiring, stocking, and using large numbers of antigen preparations for performing patch or prick skin tests and for providing allergen-based hyposensitization immunotherapy. They also use various immunoassays to measure total IgE and allergen-specific IgE in patients to assist therapeutic procedures.

Immunotherapy is often chosen by patients to treat severe allergic rhinitis and venom anaphylaxis (Ross *et al.*, 2000). A major reason is that it can sometimes achieve cure. The immunization with allergens adopted in immunotherapy drives an array of effects, including the production of protective IgG4 antibodies, the shift from TH2 to TH1, and others, which dampen the actions mediated by allergen-specific IgE (Carlsen, 2004; Ross *et al.*, 2000; Till *et al.*, 2004). The shortcomings of immunotherapy are that it does not alleviate the allergic symptoms in about half of the patients who receive the treatment and is generally not effective for treating asthma (Bousquet *et al.*, 1991; Limb *et al.*, 2006; Nelson, 2003). Another main drawback is that the administration of allergens can sometimes cause anaphylactic reactions (Bernstein *et al.*, 2004; Borchers *et al.*, 2004), especially if the doses of allergens are increased and the injection schedule is compressed. Thus, it is a rational proposition that immunotherapy and anti-IgE therapy are combined to enhance the advantages of

immunotherapy and to minimize its shortcomings (Chang, 2000). This may expand the practice of immunotherapy to cover broader patient populations (Parks and Casale, 2006).

5.1. The Combination of Anti-IgE and SIT

In four well-designed clinical studies carried out in Germany (Bez *et al.*, 2004; Kopp *et al.*, 2003; Kuehr *et al.*, 2002; Rolinck-Werninghaus *et al.*, 2004), the effects of omalizumab were investigated for augmenting the efficacy of SIT on polysensitized allergic rhinitis patients, who were allergic to birch pollens in the initial period and subsequently to grass pollens in the following period during the pollen season from approximately February to July, depending on the locations of the patients. The birch and grass pollen periods were distinctly separated by 1–2 weeks and nonoverlapping. The grass pollen antigen used in the trial was prepared by mixing six grass pollens and secale pollens. In the first segment (period) of the trial, grass antigen was used as an irrelevant antigen control, and in the second segment (period), birch pollen antigen was used as a control. The results indicated that omalizumab alone could achieve an efficacy comparable to that of SIT, and the combination of SIT and omalizumab could achieve an efficacy better than either SIT or omalizumab alone.

5.2. Priming Patients with Anti-IgE for Rush Immunotherapy

In a typical rush immunotherapy (RIT) protocol, a patient receives several antigen injections in steeply escalating doses within a few hours on the first treatment day. In some but not all protocols, the patient continues to receive several antigen injections in still sharply increasing doses within a few hours in each of the following few days in an inpatient procedure. In the following several months, the patient receives weekly increasing doses of antigen (Sharkey and Portnoy, 1996). RIT, which can be performed in an “off-season” or an “off-site,” is suggested for patients with severe allergy that needs to be treated promptly. Because the rates of increments of antigen in RIT are much more aggressive than those in a typical SIT, the incidences of severe reactions, including anaphylaxis, are inevitably much higher (Greineder, 1996). Thus, while RIT is highly desirable for resolving the allergic problems of patients in short time frames, it is highly risky.

In a recently published double-blinded, placebo-controlled clinical study investigating the utility of anti-IgE in protecting patients receiving RIT from anaphylactic reactions, the patients, who had ragweed pollen-induced seasonal allergic rhinitis, were pretreated with three doses of 4-weekly or five doses of biweekly omalizumab injections, started 9 weeks before the RIT protocol.

One week after the last omalizumab injection, patients were given six injections of Amb a 1 antigen, a major ragweed pollen antigen (100-fold increase in dosages) in 3 h or eight injections (330-fold increase in dosages) in 5 h. In the following 12 weeks, the patients received increasing doses of the ragweed pollen antigen. The results indicated that pretreatment with omalizumab could reduce the incidences of anaphylactic reactions by 80%, while significantly reducing severity scores of allergic symptoms (Casale *et al.*, 2006).

6. Pivotal Roles of IgE and FcεRI in Type I Hypersensitivity

6.1. Stages Along IgE-Mediated Allergic Pathway

Omalizumab has already been approved in many countries for treating allergic asthma and has potential for broad use in treating severe allergic rhinitis and several other IgE-mediated allergic diseases. In order to understand how anti-IgE renders its pharmacological mechanisms to achieve the effects of alleviating allergic symptoms, it is important to dissect the IgE-mediated allergic pathway and analyze the intricate interactions among its elements and related factors. Figure 5 shows that the IgE-mediated pathway can be divided into sensitization, triggering, and manifestation stages. In these three stages, the key elements are also indicated.

The sensitization stage in a patient may take many months to several years (Zeiger and Heller, 1993), depending on a complex set of intrinsic and extrinsic (environmental) factors particular to the patient. The intrinsic factors include genetic and nongenetic ones (Cookson, 1999; Novak and Bieber, 2003). In the progressive process of sensitization in a patient toward certain foreign, harmless, mostly protein antigens encountered by the patient, the B cells are influenced to switch to IgE-expressing B cells (KleinJan *et al.*, 2000). The end result of this sensitization stage is the continual production of IgE specific to these antigens, consequently creating a sensitive state, in which the patient may be triggered to mount IgE-mediated reactions on exposure to the antigens.

In the triggering stage of a patient sensitized to an allergen, the allergen-specific IgE is present at a significant proportion in total IgE (more detail in Section 6). The allergen-specific IgE occupies a significant number of FcεRI on mast cells, basophils, and activated eosinophils. These effector cells are said to be in an “armed” state and ready to “fire” anytime a threshold number of allergen-specific IgE-charged FcεRI on them is cross-linked and aggregated by allergen molecules (Ishizaka *et al.*, 1984). The triggering stage in an allergic response can be an astonishingly fast process. For example, in an allergic response to tree pollens or house dust mites, a patient takes in the pollen particles or fecal particles of mites through inhalation. The allergen particles are brought into mucous fluid in the nasal lining or lower airway and broken

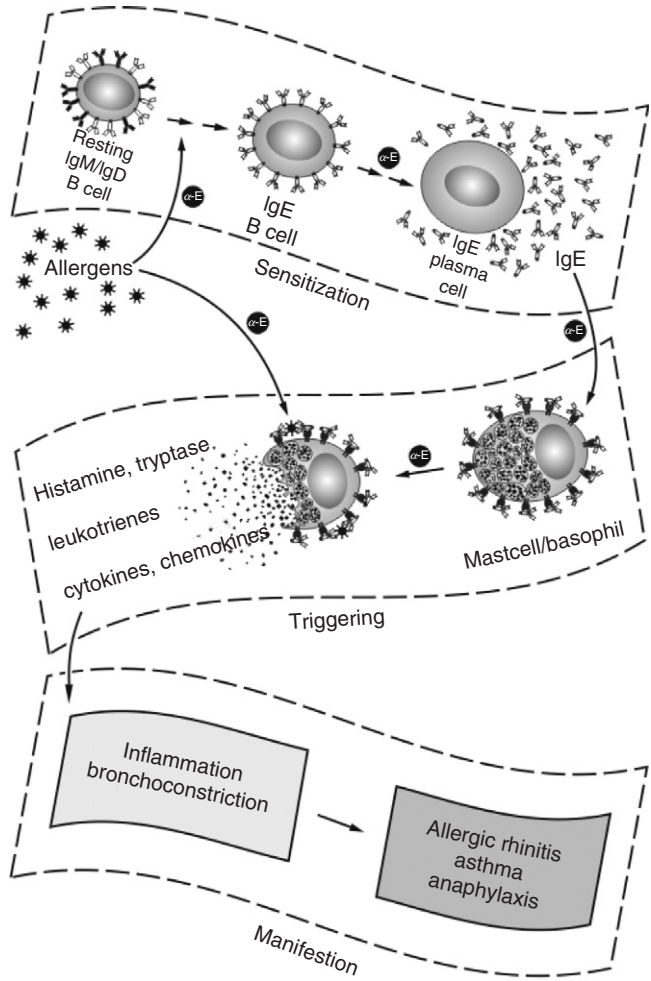


Figure 5 The IgE-mediated allergic pathway. Sites denoted by a circled α E indicated the steps where anti-IgE has been shown to act or can potentially act at.

apart and the proteins contained therein dissolved. Certain (not all) proteins find ways to get across the mucosal epithelia into the basal side, where the allergenic proteins bind to the allergen-specific IgE on mast cells, cross-linking the IgE and thereby aggregating the underlying Fc ϵ RI, initiating a signal-transducing cascade, leading to Ca²⁺ mobilization and exocytotic processes, and ultimately the discharge of pharmacological mediators from mediator-packed

granules (Janeway *et al.*, 2005; Kinet, 1999). The entire process can sometimes take less than a minute. The triggering process involving many mast cells in the local area will continue during the period a patient is exposed to over-threshold concentration of allergens.

The mediators released from sensitized mast cells and basophils are of three categories (Bingham and Austen, 2000; Serafin and Austen, 1987). The pre-packed mediators include histamines, tryptase, and chymase. In the second category are lipid mediators such as leukotrienes, which are synthesized within minutes after the sensitization of the cells. The third category includes cytokines and chemokines (Bingham and Austen, 2000; Williams and Galli, 2000), whose genes are activated and expressed as the cells are activated. These protein factors are synthesized and released after cells are activated for about 4 h.

The manifestation stage, which may last from minutes to hours to days, can be divided into periods of early- and late-phase reactions, which are mediated largely by the prepacked mediators and small molecular mediators and by the cytokines and chemokines, respectively. The mediators released from mast cells, basophils, and activated eosinophils bind to respective receptors on various cell types and cause inflammatory processes directly or indirectly, seen in all types of allergic responses. The factors also bind to smooth muscles of the airway and cause bronchoconstriction. In an anaphylaxis, a systematic, sometimes violent, reaction occurs and can pose life-threatening situations.

Numerous elements, including histamines, tryptase, leukotrienes, cytokines, and other mediators, as well as the “instability” of mast cells (Section 8.2), in the IgE-mediated allergic pathway are targets of therapeutic intervention for the purpose of attenuating allergic responses. On the basis of the pivotal role of IgE in arming mast cells and basophils, IgE is a rational target for therapeutic intervention, because blockage at this step shuts off the later steps, including the release of all mediators by mast cells and basophils.

6.2. Direct Pharmacological Effects of Anti-IgE

IgE mediates its broad range of effects via binding to FcεRI and FcεRII expressed on various cell types. The interaction of IgE with FcεRI in the IgE-mediated allergic pathway is central to almost all effects leading to the manifestation of allergic symptoms. The most direct effect resulting from the binding of anti-IgE to free IgE is that IgE is blocked from binding to FcεRI on basophils, mast cells, and activated eosinophils, which should turn off the IgE-mediated pathway shown in Fig. 5. The other direct effects are that IgE is blocked from binding to FcεRII (membrane-bound CD23) on B cells, macrophages, granulocytes, platelets, and many other cell types and to soluble CD23 in the blood and interstitial space. How the inhibitory effect on IgE binding to

FcεRI will disarm the FcεRI-equipped effectors must be assessed in terms of extent and timing as to be discussed in [Section 8](#). How blocking IgE binding to FcεRII will affect the activity of the IgE-mediated allergic pathway and overall immune activity will be discussed in [Section 11.2](#).

7. Neutralization of Free IgE

Because IgE is a key molecule in the IgE-mediated allergic pathway, neutralizing IgE and decreasing its synthesis would seem to be a logical approach to mitigate the IgE-mediated allergic pathway. In applying anti-IgE to neutralize the activity of IgE in the blood and interstitial space in a patient, a question stands out: to how low should the free IgE be brought down? In a few early clinical trials, answers in terms of the percentages of the baseline levels of plasma IgE were provided. However, those data are probably not correct, because the immunoassays for determining free IgE involved high dilution of plasma samples, which would liberate IgE from the immune complexes, resulting in an overestimation of the free IgE levels. In addition, in considering the great variations of total IgE and allergen-specific IgE concentrations among different patients, there is probably no simple answer. This section intends to analyze these issues in detail.

7.1. Total IgE and the Proportion of Allergen-Specific IgE

Anti-IgE targets the entire isotype of IgE in a patient. Now that anti-IgE is known to be capable of achieving therapeutic efficacy in treating IgE-mediated allergic diseases, one would logically ask to how low IgE should be reduced for causing a significant extent of loss of sensitivity of mast cells and basophils. The sensitivity of mast cells and basophils is a function of a host of factors. This chapter proposes that among these factors, the most critical ones are (1) the density of FcεRI occupied by allergen-specific IgE on the surface of mast cells and basophils and (2) the concentration of allergens in the fluid surrounding the cells (at the particular tissue site and at the moment of concern). Furthermore, the density of FcεRI occupied by allergen-specific IgE on the surface of mast cells and basophils are a function of two variables: (1) the proportion of allergen-specific IgE in total IgE and (2) the concentration of total IgE.

Different IgE molecules bind through their common Fc to FcεRI with an equal affinity, regardless of their antigenic specificities. Over time, the proportion of FcεRI that is occupied by allergen-specific IgE is the same as that of this allergen-specific IgE in total IgE. In the other part of the equation, the serum IgE concentration correlates with the density of FcεRI (occupied by IgE) on basophils. In a classical study published by Lichtenstein and colleagues in the

late 1970s (Malveaux *et al.*, 1978), it was found that among 26 donors analyzed, the serum IgE concentrations ranged from 2.6 to 5500 ng/ml (about 2000-folds in range), and the numbers of surface IgE molecules per basophil ranged in a well-correlating fashion from 6000 to 600,000 (about 100-folds in range). Thus, the density of FcεRI occupied by allergen-specific IgE on the surface of basophils and probably mast cells are determined by the above two variables.

Parenthetically, the above concept provides a molecular basis for explaining the “hygiene hypothesis,” which states that people who lack frequent microbial infection during infancy and early childhood, as the result of much improved hygienic conditions, have increased likelihood of developing allergic diseases. A popular explanation is that microbial infections during the first years of life condition the immune system toward the TH1 mode and hence weaken the immune activities stimulated by IL-4, IL-5, and other drivers of the TH2 branch, and conversely, in the absence of frequent microbial infections the immune system is skewed toward the TH2 mode. It is now established that IgE is involved in a dominant fashion in the pathogenesis of allergic asthma, allergic rhinitis, and probably several major allergic diseases. Therefore, the hygiene hypothesis must be explained in terms of how the IgE-mediated allergic pathway is enhanced in some individuals, who had few infections in early childhood. We believe that a plausible molecular elucidation of the hygiene hypothesis can be made by analyzing how the decrease of infections by microorganisms, viruses, and helminthic worms, and the increase of exposure to harmless environmental antigens affect in patients the total IgE concentration, the proportion of allergen-specific IgE in total IgE, and the concentration of allergens in the body fluids the inflammatory cells encounter.

7.2. IgE Concentration *versus* IgE Occupancy of FcεRI

The density of allergen-specific IgE-occupied FcεRI on mast cells will be reduced, if the total density of FcεRI is reduced. Figure 6 examines the percentages of FcεRI on a mast cell that are occupied by IgE over a broad range (5 logs) of IgE concentrations, from 0.1 to 10,000 IU/ml. This analysis indicates that in almost the entire range of IgE concentration present in the human population, the FcεRI are essentially all occupied. Only at the extremely low range of IgE levels, the percentage of FcεRI occupancy is reduced significantly: at 3 IU/ml of IgE, the proportion of occupied FcεRI is about 25%; at 1 IU/ml of IgE, the occupancy of FcεRI is about 10%. This suggests that for patients with baseline IgE levels higher than the average 300 IU/ml of asthmatic patients, IgE levels must be reduced to more than 99% for the receptor occupancy to fall below 25%. In Section 8, the stability of IgE-occupied and -unoccupied FcεRI and the turnover of FcεRI will be discussed.

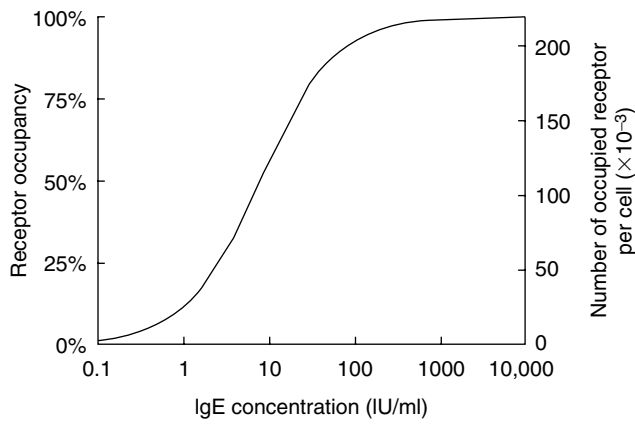


Figure 6 The relationship between IgE concentration and occupancy rate of FcεRI on basophils. The calculation is based on K_d of 1×10^{-10} M for anti-IgE's binding to IgE and 220,000 FcεRI (receptors) per basophil.

8. Downregulation of FcεRI

8.1. The Dynamical Relationship Between Free IgE and FcεRI

The above analysis that IgE must be reduced to $>99\%$ in order for FcεRI on mast cells and basophils to become significantly unoccupied is based on steady state kinetic properties, reflecting the concentration of IgE and the density of FcεRI and the interaction between them. However, on the surface of living mast cells and basophils, the presence of IgE-unoccupied and -occupied FcεRI is highly dynamic and regulated, and not merely dictated by the chemical kinetics between IgE and FcεRI.

As discussed in [Section 7.1](#), the density of FcεRI on basophils is related to the concentration of IgE in the blood ([Malveaux et al., 1978](#)). The meticulous research carried out by the groups of MacGlashan, Casale, and others on the effects of anti-IgE on downregulating FcεRI on basophils ([Lin et al., 2004](#); [MacGlashan et al., 1997](#)), mast cells ([Beck et al., 2004](#)), and dendritic cells ([Prussin et al., 2003](#)) has provided insight into the dynamical relationship between IgE and FcεRI. New molecules of FcεRI are being synthesized and routed to the surface by basophils (used in most studies), and FcεRI molecules on the cell surface are rerouted back (internalized) and degraded. On the cell surface, FcεRI are occupied by IgE according to the kinetics of binding (K_d , k_{on} , and k_{off}) between IgE and FcεRI. Statistically, when the IgE concentration is not limiting, a bare FcεRI is occupied by IgE swiftly and does not stay

unoccupied for a long time. When the IgE concentration becomes limiting, a bare FcεRI, which is freshly synthesized and routed to the cell surface or has lost its bound IgE through thermodynamic process, may not be reoccupied by IgE for a long time (MacGlashan *et al.*, 1998, 1999).

As a part of the mechanism evolved to regulate the concentration of FcεRI on basophils, IgE-occupied FcεRI are stable and maintained on the surface. FcεRI that are not occupied by IgE are structurally unstable and recognized and internalized for degradation (Kubo *et al.*, 2001). Thus, as free IgE is reduced to under a certain concentration, the replenishment of newly synthesized FcεRI does not compensate for the degradation of FcεRI, resulting in a gradual loss of FcεRI on the cell surface, until a balance between replenishment and degradation is reached.

The kinetics of maintaining FcεRI on basophils is further compounded by the fact that basophils have a life span of 1–2 weeks. As old basophils die, all FcεRI are degraded; as new basophils are generated via the differentiation of precursor cells, FcεRI are synthesized. However, if IgE concentration is very low, the synthesized FcεRI on the newly generated basophils are not occupied and are degraded rapidly, leaving a low number of FcεRI on the cell surface (MacGlashan, 2004). Unlike basophils, mast cells have a longer life span of several weeks to several months (Fodinger *et al.*, 1994), hence the renewal process of these cells plays a lesser factor in the kinetics of FcεRI maintenance on the cells (Borkowski *et al.*, 2001).

8.2. Anti-IgE as a Mast “Cell-Stabilizing” Agent

A subcutaneous injected anti-IgE antibody (humanized IgG1) should diffuse into the vasculature and get dissipated via the blood circulation and distributed in various tissues in the body within a few days. On the basis of the high affinity of anti-IgE for binding to IgE, if anti-IgE is provided in large excess over the free IgE in a person, the free IgE will be reduced to more than 99% in hours or within 1–2 days (Corne *et al.*, 1997). Such a depletion of free IgE should initiate the gradual loss of FcεRI on basophils and mast cells via the molecular and cellular dynamics discussed in Section 8.1. FcεRI on basophils are reportedly reduced by 70% in 2 weeks and by more than 97% in 3 months (Lin *et al.*, 2004; MacGlashan *et al.*, 1997). The downregulation of FcεRI on mast cells follows slower kinetics. Nonetheless, mast cells in patients treated with anti-IgE gradually lose their sensitivity to allergen stimulation, as much higher amounts of allergen are required to induce a positive skin prick test (Beck *et al.*, 2004).

In the IgE-mediated allergic pathway, mast cells are the major source of pharmacological mediators in various allergic responses, perhaps most vividly

displayed in the nasal linings, the lower airway, conjunctiva, and skin, as observed in allergic rhinitis, asthma, and atopic dermatitis. Thus, a general hyposensitization of mast cells by “mast cell-stabilizing” agents (a somewhat imprecise term, for it assumes that the mast cells in the patients are unstable) has been an attractive therapeutic approach for decades. Since as early as in the 1970s, a class of molecules, known as cromones, including disodium cromoglycogate and sodium nedocromil, has been used clinically and being investigated to treat asthma, allergic rhinitis, and allergic conjunctivitis. Cromones have been shown to be able to retard the processes of exocytosis, degranulation, and lipid mediator synthesis induced by allergen-initiated, FcεRI-mediated cascades (Edwards, 2005; Storms and Kaliner, 2005; van Cauwenberge *et al.*, 2000).

In a rough sense, the mast cells in an allergic patient are either too sensitive or undesirably too “potent.” Anti-IgE, by depleting IgE, indirectly causes the loss of FcεRI on these mast cells and renders them insensitive to stimulation by allergens. Cromones, which are generally mild in action, work by modulating the lipid membrane and slowing Ca^{2+} mobilization, which is required for the exocytotic process. Thus, cromones make mast cells impotent, while anti-IgE renders them insensitive (Chang and Shiung, 2006).

8.3. How Low Should FcεRI-IgE Fall for a Mast Cell to Become Insensitive?

In relating the density of allergen-specific IgE-occupied FcεRI to the sensitivity of mast cells, one inevitably encounters the question whether there is a threshold of density of such receptors, which could be expressed in terms of a certain number per cell. In reality, there is no specific number, because the cells are exposed to a horde of factors, all of which contribute in large or small parts to determining the threshold level of allergen-specific IgE-occupied FcεRI of a mast cell. On closer examination, among the various factors, the concentration of allergens in the fluid surrounding the mast cell (Section 7.1) is probably a dominant one. This factor is obviously highly variable among patients, as they are sensitized to different sets of allergens by different degrees. The amounts of allergens in the air or food also vary at different time points. Furthermore, the mast cells located in different mucosal areas in the body also receive different amounts of allergens.

The quantification of threshold densities of allergen-specific IgE-occupied receptors has been approached in cell culture using a hapten-specific mono-specific IgE. In the estimation, when the concentration of allergens was not limited, a minimal number of 100–200 FcεRI–IgE complexes would be enough to sensitize a basophil (Maeyama *et al.*, 1986; Posner *et al.*, 2002). If allergen-specific IgE accounts for 10% of the total IgE, a minimal of 1000–2000 FcεRI molecules would be sufficient for the basophil to be sensitive to allergen

stimulation (MacGlashan *et al.*, 1997). This would suggest that under the particular set of conditions, in which allergen-specific IgE accounts for 10% of total IgE and allergen concentration is not limiting, the total IgE concentration in the particular patient must be reduced to below 0.8 IU/ml (based on Fig. 5). In an asthmatic patient with an average plasma IgE concentration of 300 IU/ml, this represents a 99.7% reduction of total IgE. It should be noted that this analysis accounts for steady state kinetics only, without considering the dynamic FcεRI regulation on the cell.

The above data might lead to the notion that the FcεRI density must fall to very low levels for the basophils and mast cells to lose sensitivity in the patients treated with anti-IgE. This notion may not be correct, because when the proportion of allergen-specific IgE in total IgE is small and the concentration of allergens in the blood or particular mucosal areas is limiting, the density of allergen-specific IgE-charged FcεRI on the basophils or mast cells may be near a threshold level, even though the total FcεRI on these effector cells have not fallen to drastically low levels.

9. Potential Beneficial Effects of IgE:Anti-IgE Immune Complexes

The precipitous drop of FcεRI on basophils and mast cells has provided a plausible, convincing explanation for the pharmacological effect of anti-IgE in improving IgE-mediated allergic symptoms. Indeed, as basophils and mast cells are rendered insensitive to allergen stimulation, the discharge of mediators by these cells on exposure to allergens will be retarded and hence manifestation of allergic symptoms greatly diminished. Can this FcεRI down-regulation effect provides the whole explanation for the pharmacological benefit of anti-IgE? In Sections 9–11, we will discuss other potential pharmacological effects of anti-IgE, which may also contribute to its therapeutic effects.

9.1. How Soon Can Clinical Improvement Be Observed?

Understanding the kinetics of the course of symptom improvement on anti-IgE treatment is obviously very important for clinicians to apply such a treatment for allergic patients. It is also important to delineate the various pharmacological mechanisms that contribute to the therapeutic effects of anti-IgE. Although a large number of trials have been performed, there has not been a detailed analysis on the kinetics of improvement, especially in the initial weeks of anti-IgE treatment. In the unpublished first Phase II trial of anti-IgE, namely, the trial of CGP51901 on mountain cedar pollens caused allergic rhinitis in Central Texas in 1994–1995, weekly symptom severity scores were

obtained and plotted. This was possible because the trial design adopted a weekly injection schedule and allowed the study investigators to examine the trial patients on weekly time points. There was a significant drop in the overall severity score 1 week after the first injection of anti-IgE. These results were consistent with the observations made in another clinical study on allergic rhinitis, in which patients were found to make reduced response to allergen challenge within 7–14 days after the initial treatment with omalizumab (Lin *et al.*, 2004).

A detailed analysis on how soon clinical symptoms improved in asthmatic patients treated with anti-IgE was also performed, although the trial design provided mostly 4-weekly data. It was found that among the patients who eventually responded to omalizumab, 61% in 4 weeks and 87% in 12 weeks showed significant improvement (Bousquet *et al.*, 2004). In the case report of three patients with recalcitrant atopic dermatitis (Section 4.4), the patients responded favorably within 2 weeks of omalizumab treatment (Lane *et al.*, 2006). In the case report of one patient with interstitial cystitis (Section 4.4), the patient improved immediately after the first anti-IgE injection (Lee *et al.*, 2006). In the case study of one patient with cold-induced urticaria (Section 4.4), the patient improved after the second biweekly anti-IgE injection (Boyce, 2006).

It is possible that the molecular and cellular pharmacological mechanisms of anti-IgE progress in a similar time frame in patients with allergic rhinitis, allergic asthma, or other IgE-mediated allergic diseases. However, the broader involvement of inflammation and tissue damages in modest-to-severe asthma may take longer time to heal and to become clinically evident.

It was found in the study by Lin *et al.* (2004) that 1 week after the subcutaneous injection of omalizumab, the basophils in the treated allergic rhinitis patients, who were sensitive to ragweed pollens, lost about 70% of the Fc ϵ RI on cell surface. It is conceivable that the mast cells in the nasal lining of these patients should not have lost as much Fc ϵ RI as basophils. However, a nasal antigen challenge test indicated that the allergic response in the nasal lining had already been attenuated at day 7, suggesting that the mast cells in the area had already been attenuated.

Could the loss of less than 70% of Fc ϵ RI on mast cells render those cells insensitive? One would reason that the observed 70% loss of Fc ϵ RI should not be sufficient for the mast cells in the nasal lining of those patients to lose sensitivity to respond to inhaled allergens. The counts of pollen particles, such as those of ragweed and birch, per unit volume per day of air may rise 5–10 times in a 1-week period during the pollen season of a plant type (information from daily pollen counts reported in metropolitan areas). If an allergic rhinitis patient already responds to the specific pollens and is symptomatic in the

beginning of the week, and fails to respond to the pollens at 5–10 times the concentration at the end of the week due to a treatment, the 70% drop in Fc ϵ RI on those mast cells is not the likely mechanism accounting for the aforementioned attenuated allergic response. Chang (2000) presented a rational hypothesis that other pharmacological mechanisms contribute to the therapeutic effects of anti-IgE, especially in the early stages of the treatment.

9.2. The Rapidly Accumulated Immune Complexes May Serve as Antigen Trappers

The half-life of IgE in humans is 1–2 days, that of anti-IgE (a human IgG1) about 21 days, and that of anti-IgE:IgE complexes about 20 days (Fig. 2; Fox *et al.*, 1996; Lanier, 2003). The administration of anti-IgE either subcutaneously or intravenously brings free IgE to near zero concentrations within a few days. However, the IgE-secreting plasma cells, which are not targets of anti-IgE and have a life span of several months or longer, continue to secrete IgE (Section 10.1). This causes a rapid accumulation of anti-IgE:IgE immune complexes to 5–10 times the baseline levels of IgE within a week (Corne *et al.*, 1997; Milgrom *et al.*, 2001). One IgE molecule has two antigenic sites for anti-IgE and can be bound by two anti-IgE molecules at the same time; one anti-IgE molecule has two antigen-binding arms and can bind to two IgE molecules at the same time. It is hence peculiar (and scientifically fascinating) that anti-IgE and IgE form small complexes both in the test tubes and in the blood of patients treated with anti-IgE, with the largest being a hexamer complex, formed by three anti-IgE and three IgE molecules (Liu *et al.*, 1995). The anti-IgE:IgE complexes are soluble and do not precipitate in the kidney and do not cause immune complex problems (Fox *et al.*, 1996).

Both anti-IgE (an IgG) and IgE are freely diffusible across the vascular capillaries and should equilibrate between the vascular and extravascular spaces. The immune complexes are stable, owing to the high binding affinity of anti-IgE for IgE, cannot cross blood capillaries, and should remain in the blood circulation or local tissue sites, where they are formed. Thus, the anti-IgE:IgE immune complexes will be accumulated to high concentrations in the blood or in local tissue sites such as in the mucosal linings of the nasal passage and the lower airway.

The IgE comprising the immune complexes have their antigen-binding sites available for binding to antigens. Because the IgE can no longer bind to Fc ϵ RI, it should function as a blocking, protective IgG. In this way, anti-IgE converts potentially adverse, effector cell-sensitizing IgE molecules into beneficial, antigen-blocking IgE species. Thus, the rapidly accumulating immune complexes probably can serve as antigen-sweeping agents. As the allergen molecules

break through the mucosal epithelia and get into the interstitial fluid on the basal side, they are bound by the immune complexes, before reaching to IgE bound by Fc ϵ RI on mast cells residing in the area. While direct experimental evidence has yet to be obtained, it is a rational hypothesis that anti-IgE:IgE immune complexes should contribute to the therapeutic effects of anti-IgE within the first and second weeks after the first anti-IgE injection. It remains a curious question as to whether on anti-IgE treatment patients with high IgE levels fare better in the first 1 or 2 weeks than patients with much lower IgE levels.

In the off-label study of omalizumab on three patients with atopic dermatitis (Krathen and Hsu, 2005) discussed in Section 4.4, one of the patients had concomitant severe asthma and a baseline IgE level of 24,400 IU/ml. A regimen of 450 mg of omalizumab every 2 weeks for 4 months did not relieve her atopic dermatitis, but her asthma was significantly improved after starting omalizumab. It was estimated that the patient had a total of ~290-mg IgE in her body and synthesized ~145-mg IgE every 1–2 days. Clearly, dosing of anti-IgE at 450 mg every other week cannot neutralize all of the IgE in her body. Also, even a 99.9% drop in total basal IgE would still leave 24 IU/ml, which is sufficient to charge and maintain normal levels of Fc ϵ RI on basophils and mast cells. These numbers suggest that the patient's asthma was improved by mechanisms other than the depletion of IgE and the downregulation of Fc ϵ RI. Perhaps, the effect of immune complexes is one of those mechanisms.

The immune complexes may possibly function in another aspect. The trapping of incoming allergens by the immune complexes should prevent the allergens from interacting with the mIgE expressed on mIgE-committed B lymphoblasts and memory B cells. This neutralization of the antigen should inhibit allergen-driven, IgE-destined immune response (Hamelmann *et al.*, 1997; Takhar *et al.*, 2005) and hence have long-term attenuating effects on allergic response.

10. Can Anti-IgE Modulate IgE-Committed B Lymphoblasts and Memory B Cell?

One of the most intriguing issues that remain to be addressed definitively is whether anti-IgE can modulate mIgE-expressing B cells. This question is of great interest, because if anti-IgE can inhibit mIgE-committed B memory cells or B lymphoblasts, the generation of allergen-specific IgE that is induced by the new exposure to allergens should be intercepted, resulting in a profound attenuation of the allergic pathway. Several lines of *in vitro* and animal model studies amply support that anti-IgE can inhibit IgE production by B cells and cause the lysis of IgE-expressing B cells (Davis *et al.*, 1993; Haak-Frendscho

et al., 1994). In a cell culture system, where human peripheral mononuclear cells were driven by IL-4 and other factors to increase IgE-committed B cells, so that the ϵ chain mRNA expression and IgE production became measurable, anti-IgE (CGP51901) inhibited the synthesis of ϵ chain mRNA and IgE. In an *in vivo* mouse model, in which BALB/c mice were transplanted with cells of SE44 cell line (Sun *et al.*, 1991), which was an NS0 myeloma that had been transfected with human ϵ chain DNA and expressed human mIgE on surface, TESC-21 (mouse MAb anti-IgE, see Section 1.1) stopped SE44 tumor growth (Davis *et al.*, 1993). Studies by other groups also showed in mice that endogenously produced syngeneic anti-IgE antibodies could suppress IgE synthesis and decreased IgE-secreting plaques, probably by eliminating IgE-expressing B cells before they matured to plasma cells (Haba and Nisonoff, 1994). In contrast to the abundant results obtained in animal model and *in vitro* studies, similar potential effects of anti-IgE have not been revealed resolutely in human patients.

That mIgE is part of the B cell receptor on IgE-committed B cells explains the *in vitro* and animal model results. Studies with IgM-expressing B cells also demonstrate that anti-IgM antibodies, in the absence of costimulatory factors, cause apoptosis (Chan *et al.*, 1990; Mayumi *et al.*, 1996) and mediated antibody-dependent cell cytotoxicity (Walker *et al.*, 1985) and complement-mediated cell lysis of the IgM-expressing B cells (Carau and Weigle, 1983a,b). The possible decrease of IgE synthesis has been difficult to observe *in vivo* in humans because the long-living IgE-secreting plasma cells, which express very low levels of mIgE and are not targets of anti-IgE, in the bone marrow and other lymphoid tissues continue to secrete IgE (Shapiro-Shelef and Calame, 2005). Thus, even if the generation of allergen-specific mIgE-expressing B cells and IgE-secreting plasma cells is interrupted, the effect is masked by the production of total IgE.

The inhibition or downregulation of mIgE-expressing B cells by anti-IgE is suggestive in some observations. In the case report of an interstitial cystitis patient treated with anti-IgE (Lee *et al.*, 2006; Section 4.4), the initial baseline total serum IgE concentration was 653 IU/ml. Seven months after the anti-IgE treatment, the total IgE (the sum of IgE in immune complexes and free IgE) decreased to 168 IU/ml. This drop of IgE suggests that existing IgE-secreting plasma cells were gradually dying off and new plasma cells were not replenished. In the clinical study of anti-IgE on 225 pediatric patients with asthma, it was found that total IgE increased to multiples of baseline levels and then gradually declined after 16 weeks of anti-IgE treatment (Berger *et al.*, 2003). In another trial, a significant proportion of patients had IgE levels after 6 months of treatment lower than their baseline IgE levels before omalizumab (Lanier, 2006). These data indicate that at least in some patients, the

generation of new IgE-secreting plasma cells appears to be inhibited (Infuhr *et al.*, 2005).

11. Other Immunoregulatory Effects of Anti-IgE

11.1. Anti-IgE Should Neutralize the Cytokinergic Properties of IgE

IgE is one of the five classes of antibodies. Its overall structure is very similar to that of antibodies from other classes and its antigen-binding domains are from the pool of variable regions shared by other classes of antibodies. Its production is induced by exposure to antigens, albeit preferentially by parasitic worms (Capron *et al.*, 1987) and a wide range of environmental antigens. The immunological mechanisms of IgE leading to the protective and pathological effects are initiated and aggravated by its interaction with the respective antigens. Thus, in almost all regards, IgE is a bona fide antibody class that has evolved to bear a set of immune defense functions.

Aside from the ability to interact with antigens for initiating immunological processes, IgE has a characteristic that is strikingly different from those of the other antibody classes—it can bind to its receptor (FcεRI) with very high affinity by itself without prior engagement with its antigen (Metzger *et al.*, 1986). This binding to the receptor initiates an array of effects on mast cells without antigen participation, thus enabling IgE to function like a cytokine-like substance and exhibit the following cytokinergic properties. Studies by several research groups have shown that IgE alone can initiate FcεRI-mediated signal-transducing cascade (Kawakami and Kitauro, 2005), leading to Ca^{2+} influx (Lam *et al.*, 2003; Pandey *et al.*, 2004; Tanaka *et al.*, 2005), histamine release, leukotriene synthesis and release, and synthesis of IL-6 and other cytokines (Kalesnikoff *et al.*, 2001; Kohno *et al.*, 2005), although such activities are much less intense than those induced by allergens and IgE combined. As for the effects on the survival (Asai *et al.*, 2001; Kalesnikoff *et al.*, 2001; Kawakami and Galli, 2002), histamine content (Tanaka *et al.*, 2002), and migratory activity of mast cells (Kitauro *et al.*, 2005), IgE's effect is no less than that of IgE-allergen complexes. Thus, IgE by itself potentiates mast cells in inflammatory processes (Kawakami and Kitauro, 2005).

Monoclonal IgE antibodies have been shown to be either highly or poorly cytokinergic. The difference appears to lie in the variable domains (Foote, 2003; James *et al.*, 2003) and the molecular mechanisms concerning such a baffling difference remain to be elucidated. The native polyclonal IgE as a whole in a human body resembles highly cytokinergic IgE (Kitauro *et al.*, 2003). By virtue of its ability to bind to IgE and block IgE binding to FcεRI, anti-IgE can impede the various cytokinergic activities of IgE, damping the inflammatory makeup in the immune system.

11.2. The Overall Attenuation of Immune Reactivity

The IgE-mediated allergic pathway, which generates mediators manifesting allergic symptoms, flames up the immune activities broadly in the local areas or systematically. The wide spectrum of cytokines, such as TNF- α , interferon- α , IL-4, and IL-5, and various chemokines, secreted by mast cells and basophils are inflammatory in nature. These factors not only cause symptoms directly but also activate and recruit various cell types augmenting the inflammatory state. Anti-IgE, by tying up IgE, eventually attenuates the IgE-mediated pathway and hence the inflammatory conditions. Such an inhibitory effect can even be observed by the overall decrease of T and B cells in the immune system (Holgate *et al.*, 2005b; Noga *et al.*, 2003; Ong *et al.*, 2005).

CD23 on cell surface and soluble CD23 play multiple roles in regulating IgE synthesis and in inducing immune response to allergens. IgE:allergen complexes bind to membrane CD23 (via Fc of IgE) and facilitate antigen presentation (Getahun *et al.*, 2005; Kilmon *et al.*, 2004). Simultaneous binding of soluble CD23 to mIgE and CD21 on B cells will lead to the enhancement of IgE production (Aubry *et al.*, 1992; Reljic *et al.*, 1997). It has been found that in the gastrointestinal tract, CD23 on mucosal epithelial cells can transport IgE from the basal side to the lumen through transcytosis (Tu *et al.*, 2005). This IgE in the lumen binds to food-derived antigens and facilitates its entry across the mucosal epithelial layer (Li *et al.*, 2006). Anti-IgE blocks IgE binding to CD23 and hence inhibits many of these processes.

The depletion of free IgE by anti-IgE has a profound, fast effect on down-regulating Fc ϵ RI on dendritic cells (Prussin *et al.*, 2003). Seven days after administering anti-IgE, the Fc ϵ RI on both subsets of precursor dendritic cells were already decreased significantly (estimated 30–50%). This should inhibit the antigen-presenting process going through IgE and dendritic cells (Kraft *et al.*, 2001; Novak *et al.*, 2003b).

11.3. Local Environment in Disease-Affected Tissues is of Utmost Interest

Much of our understanding on the dynamic interactions between IgE and anti-IgE has been derived from studies analyzing such interactions in the blood. However, mast cells, which are key players in allergic rhinitis, asthma, dermatitis, reside in extravascular space in disease-affected tissues (Brody and Metcalfe, 1998; Liu, 1997). It is believed that mast cells establish residency in specific locations and develop different sets of characteristics (Church and Levi-Schaffer, 1997). It is conceivable that mast cells residing in the nasal lining, lower airway, other areas of the mucosal tracts, and in the skin, differ in tryptase and chymase content, sensitivity, receptor regulation, and life span (Chang and Shiung, 2006; Galli and Hammel, 1994; Irani and Schwartz, 1994;

Lowman *et al.*, 1988; Miller and Schwartz, 1989; Peng *et al.*, 2003). Interestingly, clinicians participating in the anti-IgE clinical trials observed that the symptoms of allergic rhinitis or allergic asthma were improved much sooner than skin prick test reactivity (Beck *et al.*, 2004). Are the mast cells in the airway and nasal mucosal linings different from those in the skin (Pawankar *et al.*, 1997)?

The local microenvironment in the allergic disease-affected tissues is of great interest, awaiting vigorous investigation. Airborne allergen particles are trapped at the mucosal surfaces in the nasal lining and the bronchial tracts and the allergen molecules contained therein find ways to get through the mucosal epithelia to the basal side. Here the allergen molecules interact with many cell types, including mast cells, dendritic cells, and probably IgE- and IgG-committed lymphoblasts and memory B cells. It is now known that allergen-specific IgE-secreting plasma cells in the local mucosa exist (KleinJan *et al.*, 2000; Smurthwaite *et al.*, 2001) and that allergen-specific B cells are driven by allergens to switch to IgE-expressing B cells and to undergo affinity maturation (class switch recombination) *in situ* in the mucosa (Coker *et al.*, 2003; Tanaka *et al.*, 2005; Wilson *et al.*, 2002). The IgE secreted by those plasma cells in the local area can sensitize mast cells in the same site.

On subcutaneous injection of anti-IgE, the anti-IgE molecules diffuse into the vascular space and are dissipated by the blood circulation. They diffuse into the local microenvironment of the affected tissues and bind to free IgE, forming immune complexes, which can no longer diffuse into the vascular space. Because the locally synthesized IgE may be rich in allergen-specific IgE, the accumulated immune complexes should contain IgE that can help capture the incoming allergens (Section 9.2).

12. Can Anti-IgE Attain a Long-Term Remission State?

The value and impact of the anti-IgE therapy will be greatly expanded, if it can achieve a long-term remission state at least in some of the treated patients. The clinical trials performed so far have not incorporated a study segment to address this aspect for a few considerations. A major concern among officials at governmental regulatory agencies and researchers developing the anti-IgE program during the early phase of the clinical development of anti-IgE was that the effect of anti-IgE in depleting IgE is not reversible. Since the initial concept was to use anti-IgE to target or deplete IgE-expressing B cells and since IgE was believed to be required for certain immune defense functions such a concern was logical. Now that the depletion of IgE for 1 or 2 years is known not to pose great risks (Ayres *et al.*, 2004; Berger *et al.*, 2003; Finn *et al.*, 2003; Lanier *et al.*, 2003), we should address whether anti-IgE can (1) have

long-term effects on the functions of IgE and (2) attain long-term remission state in the treated patients.

Information concerning whether anti-IgE can attain a long-term remission state originates from anecdotal, nonsystematic observations made by clinicians treating asthma patients. Clearly, some patients appeared to have achieved remission state. However, there are no statistical data on the proportion of cases and the duration of such remission states. There is no information as to what makes an anti-IgE treatment to achieve a remission state. Nonetheless, anti-IgE, when used in chronic protracting allergic asthma, helps tame an inflammatory state and tune-up or rebuild a healthy airway.

If anti-IgE can drive toward a remission state, it probably causes a shift in the balance between IgE-mediated and non-IgE-mediated responses. Many pharmacological mechanisms of anti-IgE should contribute to such a shift. Among them, potentially the most potent one is the modulation and downregulation of IgE-committed B cells by anti-IgE, as discussed in [Section 10](#). In a patient, the generation of IgE-committed allergen-specific memory B cells is an important end result of the sensitization process that may take as long as a few years ([Weissman and Lewis, 2002](#)). Thus, if anti-IgE can downregulate or eliminate IgE-committed B memory cells, it should have very profound, long-term attenuating effects on the state of sensitivity against allergens.

When a patient is under anti-IgE treatment, the IgE-mediated allergic pathway is eventually blocked off and the numerous IgE-related activities discussed in [Sections 9–11](#) inhibited. Since the patient is exposed to allergens as usual, the allergens should induce other immune responses, such as protective IgG, against the allergens. These responses should gradually drive a shift in the balance between IgE versus non-IgE-related responses toward the non-IgE compartments. When a substantial shift is created, the patient should achieve a remission state. Along this line of rationalization ([Chang, 2000](#)), since the shift to favorable non-IgE-related responses may be induced by allergen-based immunotherapy (SIT or RIT, discussed in [Section 5](#)), a combination of anti-IgE with SIT or RIT may help patients achieve long-term remission state or even cure more effectively and safely than with SIT or RIT alone.

13. Are There Adverse Effects Associated with Anti-IgE Therapy?

13.1. Is Immune Defense Function Compromised?

It is a rational assumption that the IgE antibody class had evolved in a branch of the vertebrate species for immune defense ([Warr *et al.*, 1995](#)). While there is not a large body of evidence in the literature supporting IgE's roles in immune defense, a significant number of papers suffice to indicate that IgE contributes in part to the defense of various infectious agents, especially

parasitic worms, in many animal species. Because immunity against infectious agents is critical for the survival of many animal species, the immune system has evolved to be highly redundant, so that multiple immune mechanisms corroborate to defend against an invading infectious pathogen (Litman *et al.*, 2005). Thus, IgE is probably still essential for animals and even humans living in primitive habitats. However, for humans living in many regions of the world today, or for pets, laboratory animals, even live stocks housed in relatively clean, confined facilities, IgE appears to have become nonessential (Lanier and Chang, 2004). This understanding is supported by the following research findings.

The roles of IgE in immune defense, especially against parasitic worms, have been studied in various types of laboratory mice, including wild-type ones producing normal ranges of IgE and biologically or genetically manipulated ones, which are devoid of either IgE or IgE-mediated effector functions. In some studies, wild-type mice were treated with anti-IL-4 antibody to abolish their ability to produce any IgE. In other studies (Madden *et al.*, 1991; Sher *et al.*, 1990), two strains of mice, which failed to produce any IgE, were employed: in one strain the gene for the IgE ϵ chain was knocked out (Gurish *et al.*, 2004; King *et al.*, 1997), while in the other strain, the IL-4 gene was impaired (El Ridi *et al.*, 1998; Watanabe *et al.*, 1988, 1993). Other studies used another strain of mice, in which the gene of the Fc ϵ RI α subunit was knocked out (Jankovic *et al.*, 1997), and IgE could not sensitize the mast cells and basophils in them. In another set of experiments, mice were treated with polyclonal antibodies that neutralize IgE (Amiri *et al.*, 1994).

Studies have been performed by a number of groups to investigate whether these mice incapable of producing IgE or devoid of Fc ϵ RI were weakened in their ability to defend the challenges of various parasites, including *Schistosoma masoni*, *Nippostrongylus brasiliensis*, and *Trichinella spiralis*. The results as a whole were inconsistent and failed to prove that a lack of IgE pathways impairs the ability to eliminate parasites. One of the anti-IgE clinical trials on asthma, which was carried out in Brazil, had monitored parasite infection as part of the study protocol. No increased rate of parasite infection was observed.

The question, whether IgE is involved in the immune surveillance of malignantly transformed cells and whether anti-IgE compromises such a function, had been raised. On the basis of the numbers of malignancies identified during the anti-IgE treatment period, it was cautioned that an apparent increase of malignancy incidences was found in the anti-IgE-treated population (Strunk and Bloomberg, 2006). Since the overall rate was within the range found in the general population, and the malignancies found in these mostly older allergy patients were of heterogeneous tissue origins, governmental regulatory agencies

did not sound an alarm for such a concern, but required that a multiyear postmarket follow-up be performed.

13.2. Observed Adverse Reactions

Aside from the concern that anti-IgE may compromise certain immune functions, anti-IgE is well tolerated and causes relatively few serious adverse effects (Strunk and Bloomberg, 2006). The various symptoms of discomfort, such as fever, headache, injection site rashes, are common for a subcutaneous injectable and for an antibody drug, since the rates of these complications were similar in patients receiving anti-IgE treatment or placebo. There have been a few cases of anaphylactic reactions among patients with very sensitive disposition and complex, multiple diseases. Anti-IgE may still benefit those highly sensitive patients, but caution must be taken while administering the drug (Dreyfus and Randolph, 2006). There has been no report of immune complex diseases or serum sickness disease. Omalizumab was found not to induce antibody response against itself.

14. Other Approaches for Targeting IgE or IgE-Expressing B Cells

14.1. Approaches for Attenuating IgE-Mediated Allergic Pathway

Various therapeutic approaches have been developed to modulate the immune system to inhibit IgE synthesis, to inhibit TH2 response, or to drive a shift from TH2 to TH1 response (Stokes and Casale, 2004). These include anti-IL-4, anti-IL-5, and IL-4 and IL-5 receptor antagonists (Barnes, 2002; Kips *et al.*, 2001; Yamagata and Ichinose, 2006). These immune modulators appear to be very attractive agents for attenuating the IgE-mediated pathway and of the TH2 arm. However, the results from clinical studies are not satisfactory in terms of their abilities to alleviate clinical symptoms. Anti-CD23 has also been studied in human clinical studies and been shown to downregulate blood IgE levels by 50% (Nakamura *et al.*, 2000; Rosenwasser *et al.*, 2003). The initial assessment was that anti-CD23 probably cannot improve clinical symptoms (Poole *et al.*, 2005).

The ineffectiveness of these various approaches may be due to their inability to downregulate IgE to a great extent. On the basis of the results of anti-IgE on neutralizing IgE and on downregulating FcεRI on basophils and mast cells, for most patients with IgE concentrations above 10 IU/ml, if the IgE concentration is not reduced by more than 95% or 99%, downregulation of FcεRI would not result. Since anti-IL-4-treated and IL-4 gene knocked out mice do not produce any IgE (Watanabe *et al.*, 1988), why can anti-IL-4 and soluble IL-4

receptor not lower IgE to near zero levels (Borish *et al.*, 2001; Hart *et al.*, 2002)? One plausible explanation is the fact that IL-4 acts at very low concentrations in short distances in the lymphoid microenvironment (de Vries *et al.*, 1993) and therefore, it requires very large amounts of anti-IL-4 to flush the local microenvironments.

14.2. An Approach to Target a Unique Epitope on mIgE

The many molecular attributes of anti-IgE, such as having a long half-life, forming small immune complexes, not inducing antibody response, and multiple proven and potential pharmacological mechanisms, are very difficult to attain with another therapeutic. However, anti-IgE is expensive and will probably not be affordable for most patients with very high IgE (Ames *et al.*, 2004) such as those with plasma IgE higher than 1000 IU/ml. Therefore, an approach that can target IgE-committed B cells directly and inhibit the synthesis of IgE substantially will be very desirable (Chang *et al.*, 1990).

IgE-committed B lymphoblasts and memory B cells express mIgE. IgE-expressing B lymphoblasts are in the differentiation and maturation process to become IgE-producing plasma B cells. In addition, mIgE is not expressed in other cell types. Thus, mIgE appears to be an ideal target for immunological agents that aim at modulating mIgE-expressing B cells. Our group had made a seemingly unlikely discovery (Peng *et al.*, 1992) that the ϵ chain of human mIgE contains a 52-amino acid peptide segment (referred to as the C ϵ mX domain) between the CH4 domain and the C-terminal membrane-anchoring transmembrane peptide (Fig. 7). Using mouse membrane ϵ chain as a reference, C ϵ mX is resulted from an alternative splicing using an acceptor site 156-base pairs upstream of the previous known site. In human mIgE, ϵ chains without C ϵ mX, while faintly expressed at the mRNA level, are not detectable at the protein level (Peng *et al.*, 1992).

The sequence of C ϵ mX shares no significant homology with sequences in the entire DNA and protein databases. Thus, the uniqueness of C ϵ mX has provided an attractive site for targeting mIgE and mIgE-expressing B cells. Because mIgE is part of the B cell receptor, antibodies targeting mIgE should activate signal-transducing process, leading to the anergization or apoptotic pathway of those cells, in the absence of costimulators (Donjerkovic and Scott, 2000; Gauld *et al.*, 2005; Tighe *et al.*, 1997). Mouse anti-C ϵ mX MAbs, such as α 20, have been prepared (Chen *et al.*, 2002) and are in the process of being humanized in our laboratory. It is possible that anti-C ϵ mX antibodies can be used in combination with anti-IgE for treating patients with very high IgE. The potential advantage of an anti-C ϵ mX antibody is that because the antibodies are not neutralized by IgE, a much smaller amount of the

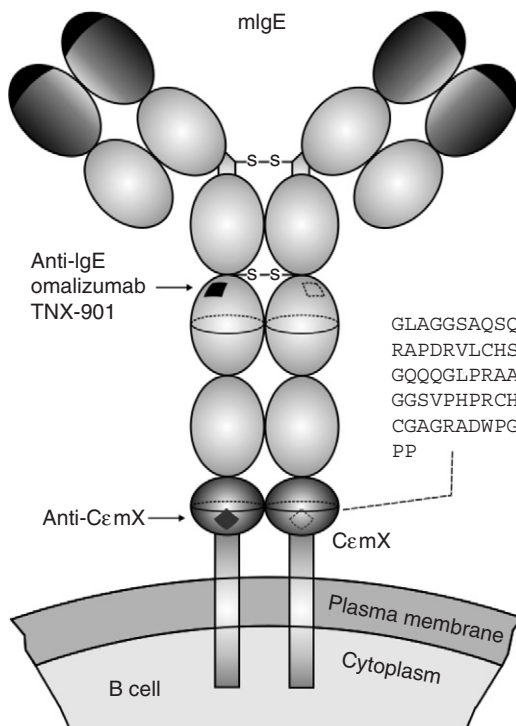


Figure 7 The location and sequence of CεmX domain in mIgE. The antigenic sites targeted by anti-IgE MAb, such as omalizumab and TNX-901, and by anti-CεmX MAb, such as a20, are indicated.

antibody will be required for each administration. If IgE-committed B cells are indeed purged by the anti-CεmX antibody, the new synthesis of allergen-specific IgE will be interrupted, resulting in a long-term attenuating effect on the sensitization state of patients.

Perhaps after a decade of use of anti-IgE, the concern over long-term depletion of IgE will subside, and a therapeutic approach for developing a vaccine-like product based on the CεmX peptide should become attractive. The CεmX peptide can be linked to a T cell reactive foreign peptide and used as an immunogen to elicit in patients antibodies that, like the passively administered anti-CεmX antibodies, bind to mIgE and modulate mIgE-expressing B cells. Such a treatment will not require large amounts of the immunogen product and may have long-term effects on suppressing IgE production.

15. Concluding Remarks

The clinical utility of omalizumab for pediatric asthma, allergic rhinitis, peanut allergy, atopic dermatitis, and others, and for combining with SIT and RIT will probably take another 5–10 years to develop for most regions of the world. The therapeutic efficacy exhibited by omalizumab and TNX-901 in about 30 Phase II and III clinical trials amply demonstrates that IgE plays significant roles in the pathogenesis of not only allergic rhinitis but also allergic asthma, peanut allergy, and probably atopic dermatitis. The clinical trial results also establish that IgE depletion is a feasible strategy for treating various IgE-mediated allergic diseases. Furthermore, the results from anti-IgE and from other experimental drugs, such as anti-IL-5 and anti-CD23, also reveal that the IgE depletion should be nearly complete and should last for a long term, at least several months.

The successful development of anti-IgE has provided a treatment option for many patients with difficult-to-treat allergic asthma and in the future, anti-IgE may also provide a treatment option for patients with other IgE-mediated allergic diseases that are severe and difficult to treat. Anti-IgE will not be an affordable treatment option for most patients in economically less developed countries. The yearly requirement of omalizumab for an asthma patient is about 2–8 g, which represents substantial production costs. Even with such sizable quantities, they are only suitable for those patients whose serum IgE levels are less than 700 IU/ml. About 10–15% of patients have IgE levels above 700 IU/ml. Thus, for economically less fortunate patients and those with high IgE, including most atopic dermatitis patients, a rather different thinking in providing anti-IgE or and on the understanding of the diseases must be stimulated. It should be noted that while excluding patients with IgE above 700 IU/ml had a rationale basis, excluding patients with IgE below 30 IU/ml did not have sound basis (Lanier, 2006). As long as their allergic diseases are IgE-mediated, those patients should respond to anti-IgE well.

While many researchers have unraveled multiple intriguing pharmacological effects of anti-IgE, we still fall short in elucidating definitively the pharmacological mechanisms responsible for the therapeutic efficacy of anti-IgE. While the downregulation of Fc ϵ RI antibodies on basophils and mast cells has been established, numerous other pharmacological mechanisms, as outlined herein (Sections 9–12) have not yet been convincingly validated. These other mechanisms very likely play important roles, especially in the first few weeks, before the downregulation of Fc ϵ RI has reached physiologically significant levels.

Among many of the questions that remain to be resolved regarding the pharmacological effects and therapeutic efficacy of anti-IgE is whether anti-IgE can modulate or inhibit IgE-committed B lymphoblasts and memory B cells.

Such an effect is critical for anti-IgE to (1) intercept the new synthesis of allergen-specific IgE, (2) cause a decisive shift from TH2 to TH1 conditions, and hence (3) achieve a long-term effect on attenuating IgE-mediated diseases. In this aspect, different patients may respond differently. Also, different anti-IgE antibodies, such as omalizumab and TNX-901, may act differently. In this regard, the development of an anti-IgE antibody with an affinity much higher (by say 100-fold) than omalizumab and TNX-901 will be of great interest. Thus, while applications based on the anti-IgE concept has been in active development for nearly 20 years, much research is needed to address the many questions posed herein.

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