

Treatment of allergic airways disease with anti-IgE

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The discovery in 1921 by Carl Prausnitz and Otto Küstner that the serum from an atopic individual could mediate local immediate hypersensitivity to a specific allergen at the site of passive transfer and fixation was to start a determined search for the underlying basis of allergic diseases such as asthma, hay fever, and food allergy (1). The identification of this “reagin” as the immunoglobulin IgE by Johansson & Bennick (2) and Ishizaka & Ishizaka (3), and the description of its capacity to bind with high affinity to activating Fc ϵ R1 receptors on mast cells and basophils (3, 4) opened up the possibility of targeting this interaction for therapy of allergic diseases. Although drugs such as sodium cromoglycate and nedocromil sodium have been developed as “mast-cell-stabilizing” agents, their potency and efficacy have proven to be relatively low.

IgE antibodies provide the triggering signal for initiating immediate hypersensitivity reactions. In the process of becoming sensitized to one or more allergens, B cells are stimulated and switched to IgE production by interleukin (IL)-4 and IL-13. This IgE binds to cells processing either or both high-affinity (Fc ϵ R1) and low-affinity (Fc ϵ R2, CD23) receptors (5). Mast cells and basophils are especially well endowed with these receptors, which provide the principal mechanism for their activation to release granule-associated, preformed mediators, newly generated lipid mediators, and a range of cytokines. Histamine, prostaglandin (PG)D₂, and the cysteinyl leukotrienes (Cyst LTs) account for most of the allergen-induced early (immediate) response, while tryptase, tumour necrosis factor-

alpha (TNF- α), IL-4, IL-13, and IL-5 provide a mechanism for the late reaction through the sequential upregulation of adhesion molecules on the microvasculature and the recruitment, followed by activation, of neutrophils, eosinophils, and T cells (6).

In view of the pivotal role that IgE has in triggering immediate-type hypersensitivity responses, its capacity to bind to its receptors represents a potentially important therapeutic target. In addition, the recent identification of both Fc ϵ R1 and Fc ϵ R2 on monocytes, dendritic cells, epithelial cells, and possibly eosinophils suggests important roles for IgE in the regulation of other aspects of the allergic response, including enhanced allergen uptake and presentation (7). Fc ϵ R1 is comprised of four subunits $\alpha\beta\gamma_2$. Careful structural studies have identified the 11 N-terminal amino-acid residues (329–340) of the C ϵ 3 domain of IgE as being essential for high-affinity binding to the α chain of the Fc ϵ R1 complex (8). Subsequent studies have demonstrated that the essential structural determinant for IgE/Fc ϵ R1 recognition depends on a consecutive sequence of 11 amino acids (343–353) in C ϵ 3 computed to form a loop structure (A–B) at the interface of the C ϵ 4 domain, and comprising a “ridge” with further interactions involving the C–D and D–E loop structures (see below).

Antibodies that bind to the Fab portion of cell-bound IgE have been used as a means to activate human mast cells for many years (reversed anaphylaxis), since, like allergen, it achieves dimerization of Fc ϵ R1, the principal activating signal for mast

cells and basophils. The idea of an inhibitor of IgE binding to Fc ϵ R1 but which lacks the ability to trigger degranulation of IgE-sensitized cells has been realized by generating a monoclonal antibody (mAb) which binds IgE at the same site as the α chain of Fc ϵ R1. As a consequence of this specificity, the antibody has the inherent capacity to inhibit IgE effector functions by blocking IgE binding to Fc ϵ receptors on proinflammatory cells and, at the same time, ensures that the antibody does not produce IgE cross-linking (9).

In 1981, Hook et al. (10) first described a mAb to human IgE that failed to elicit histamine release, and they correctly concluded that the determinant recognized on IgE was excluded by the Fc ϵ R1 interaction. It was subsequently shown that similar antibodies to mouse IgE could effectively inhibit IgE binding to its receptor (11). However, these antibodies also induced receptor cross-linking. Later, antibodies to mouse IgE were isolated which inhibited IgE binding without producing IgE-receptor dimerization (12, 13), since they reacted to a single determinant within the region of IgE that recognized Fc ϵ R1. However, they could interact with IgE expressed on the surface of B-hybridoma cells.

While these studies were proceeding, the existence of naturally occurring anti-IgE autoantibodies had been recognized since 1989 (14). Such antibodies, depending upon their sites of binding to IgE, could amplify or inhibit IgE-mediated responses. Anti-IgE autoantibodies have been reported in atopic dermatitis (15), Hymenoptera sting allergy (16), and asthma (17). Most recently, a high proportion (60%) of patients with chronic "idiopathic" urticaria have been shown to have antibodies against the α chain of Fc ϵ R1, while up to 10% have anti-IgE antibodies (18). Fractionation and characterization of these different anti-IgE autoantibodies show that most belong to the IgG class and may either inhibit or enhance IgE-Fc ϵ R1 binding (19).

A nonanaphylactogenic antibody to human IgE was generated by virtue of its inability to trigger histamine release from IgE-sensitized basophils (20). Two other approaches have been taken. The first was to select a mAb specific for the region of membrane IgE exposed on switched B cells (21). A second approach was to use homology scanning mutagenesis to generate a murine antibody, MAE-11, which identified the same key amino acids in the C3 domain of IgE as did the α chain of Fc ϵ R1 (22). The target structure of these nonanaphylactic murine antibodies has been studied by site-directed mutagenesis of IgE and shown to comprise six key residues, Arg 408, Ser 411, Lys 414, Glu 452, Arg 465, and Met 469, localized in three loops with C ϵ 3 to form a ridge on the most exposed portion of

human IgE (22). The binding site of IgE for Fc ϵ R2 (CD23) lies within the same region that binds to Fc ϵ R1 (5); therefore, antibodies selected for the latter also inhibit binding to CD23.

The use of anti-IgE to inhibit IgE responses was described first, in 1982, by Bozelka et al. (23), who showed that treatment of mice from birth with a polyclonal anti-IgE reduced serum IgE levels, an observation that was later extended to adult mice (24). Of particular interest in these studies was the effect that anti-IgE treatment had on reducing the number of IgE-secreting B cells; moreover, if the antibody was given during immunization, the secondary IgE response was attenuated but did not prevent induction of memory (25). The total and antigen-directed IgE in the serum fell by over 90% and this was paralleled by a similar decrease in IgE-secreting cells. This latter effect requires bivalent recognition of surface Ig and the absence of appropriate costimulatory signals (26), but it did not require T-cell help (27, 28). Treatment of mice with a single injection of an anaphylactogenic anti-IgE mAb during primary immunization reduced serum IgE, but not IgG, to undetectable levels for over 2 months, even when the animals were exposed to antigen on a weekly basis (28).

With the production and characterization of nonanaphylactogenic rat IgG1 against mouse IgE (9, 12, 13, 32, 33), it has been possible to analyse responses on mice immunity (12, 32). Treatment of preimmunized mice reduced specific IgE by over 80% without altering IgG secondary responses. Three months after treatment, IgE responsiveness had returned to normal. Systemic administration of mAb 1-5 produced partial inhibition of the skin wheal response to antigen by day 4 and total inhibition by day 5 (32, 33). This indicated that anti-IgE was not able to displace bound IgE but did abolish target cell responsiveness by blocking the binding of IgE to free Fc ϵ receptors.

The clear inhibitory effect that the anti-IgE mAb 1-5 had on mast-cell-dependent skin whealing suggested that it might also inhibit late-phase inflammatory responses produced by mast-cell cytokine release. Antigen challenge of the lungs in mice induces an eosinophilic response linked to IL-4 and IL-5 production by Th-2 polarized T cells (34, 35). Systemic administration of mAb 1-5 totally suppressed antigen-induced eosinophil recruitment (36). This depended upon an Fc ϵ R2 mechanism, because in CD23-deficient mice, or in the presence of blocking antibodies to CD23, the protective effect of mAb 1-5 on the eosinophilia was lost (36). Therefore, it was suggested that, at least in this mouse system, anti-IgE inhibited antigen-induced eosinophilia by reducing IgE-facilitated antigen presentation for T-cell stimulation (37).

Administration of anti-IgE mAb to rats before or shortly after passive sensitization with antigen-specific IgE inhibited immediate bronchoconstriction by over 90% (33, 38) and caused about 70% inhibition of the antigen-acquired bronchial hyper-responsiveness to 5-HT challenge (38). Similar results have been reported for the ability of a nonanaphylactogenic anti-IgE mAb to inhibit sensitization of both monkey and human bronchi *in vitro* (39, 39a).

With the clear indication that a monoclonal antibody that bound to the Fc ϵ R1 α -binding region of IgE could influence both immediate and late-phase allergic-type responses in animals, the stage was set to apply this to develop a therapy for use in man. There were, however, two outstanding problems: interference with protection against parasites and neutralizing immunologic responses to a mouse protein. In relation to concern over parasite immunity, it came as a surprise that anti-IgE treatment of mice infected with *Nippostrongylus brasiliensis* (40) or *Schistosoma mansoni* (41) resulted in effective elimination of parasites, decreased worm burden, and reduction in the number of parasite eggs, which paralleled the fall in serum IgE. Thus, high levels of serum IgE induced by parasites may be a peripheral consequence of strong Th-2 cell induction and increased IL-4 production, and may not be centrally involved in parasite immune defence, as previously thought.

The second issue related to immunogenicity of mouse protein. This problem was negotiated by generating both chimeric and reshaped "humanized" mouse nonanaphylactogenic anti-human IgE antibodies in the form of CGP 51901 (chimeric) (32) and CGP 56901 (humanized) (42) derived from the parental mouse antibody TES-C21 and rhu mAb E25 (humanized) derived from the MAE-11 mouse clone (17). The humanization technology, pioneered by Winter and colleagues (43), involved the grafting of the murine antigen-binding loops (the complementarity-determining regions [CDRs]) onto the human antibody framework. Selected non-human framework residues also need to be incorporated into the humanized antibody to maintain proper CDR conformation (42). Both TES-C21 and MAE-11 were humanized with a human Ig framework derived from consensus sequences of human V $_L$ and V $_H$ subgroups (45), which allows use of the most common framework found in human IgG antibodies. On this basis, CGP 51901 and E25 (which contained under 5% mouse sequences) were selected for further study, since their activity was most closely comparable to that of the parent murine antibodies.

When studied *in vitro*, these antibodies blocked the passive sensitization of human basophils and

mast cells but, importantly, failed to bind to IgE bound to Fc ϵ R1 or Fc ϵ R2 and trigger mediator release (46, 47). Like the parent mAbs, CGP 51901 and E25 recognized IgE expressed on the surface of B cells, but not IgE-sensitizer Fc ϵ R2-bearing cells (32). They were shown to inhibit IgE production by human B cells *in vitro* (32). Despite these similarities, the two humanized antibodies were directed to separate epitopes on the C ϵ 3 region of IgE. A series of safety studies showed that both antibodies bound only to B cells, not mast cells, in various tissues (48). E25 bound with comparable affinity to cynomolgus monkey (3×10^{10} M) and human IgE (1.7×10^{10} M) (49). This was further exploited *in vivo* by showing that twice-weekly i.m. injections of E25 in animals presensitized with 27 μ g of human ragweed-specific IgE resulted in a reduced skin wheal response to ragweed antigen in five out of six animals, with a 100% response rate after the second dose of E25 persisting for 1 month.

Administration of anti-IgE antibodies results in the formation of immune complexes. The immune complexes formed *in vitro* with IgE by E25 have been shown to be small by gel-filtration chromatography and analytic ultracentrifugation (50, 51). A molar equivalence, the largest complex of approximately 1×10^6 Da, predominates. The complexes are best described as a cyclic hexamer consisting of three molecules of each immunoglobulin. *In vivo* E25-IgE complexes were again small, but this time were similar to the complexes formed *in vitro* when IgE was in excess. These experiments conducted in cynomolgus monkeys showed no specific uptake in any particular tissue 1 and 96 h after administration.

A series of single and multidose phase 1 studies showed that E25 and CGP 51901 were well tolerated by human subjects and resulted in a dose-related decrease in serum levels of "free" IgE (52). After a single 100-mg dose of CGP 51901, free IgE fell by 96% with a mean recovery to 50% of baseline IgE by 39 days (52). As might be expected, total IgE comprising free and complexed forms increased, indicating slow clearance of the anti-IgE-IgE complexes. No detectable antibody response in the recipient was detected. Taken together, these studies support the view that using an anti-human IgE antibody is safe and effective at reducing IgE levels in man, and encourage further clinical development.

In the recognition that IgE is an important trigger mechanism for allergic airway diseases, the first clinical trials investigated the efficacy and safety of E25 in the allergen-induced early and late asthmatic responses. The effect of E25 on the provocation dose of allergen causing a 15% reduction in FEV $_1$ from baseline (PC $_{15}$ allergen) during

the early asthmatic response was assessed in a multicentre, randomized, double-blind, parallel-group study (53). E25 was well tolerated, and only one patient was withdrawn because of a generalized urticarial rash after the first dose. When compared with baseline values, the median PC₁₅ allergen values on days 27, 55, and 77 were increased by 2.3, 2.2, and 2.7 doubling doses, respectively, with E25 and -0.3, +0.1, and -0.8 doubling doses with placebo ($P \leq 0.002$). The methacholine PC₂₀ value improved slightly after E25, becoming significant on day 76 ($P < 0.05$).

An additional study (54) examined the effect of 9 weeks of E25 in a parallel-group, randomized, double-blind, placebo-controlled protocol with 19 mildly asthmatic patients who were atopic. E25 reduced the serum IgE to under 90% and resulted in attenuation of the maximum fall in FEV₁ during the early response from 30% at baseline to 18% after active agent, compared to values of 33% and 34% after placebo. The late-phase response was also reduced by 60%, eosinophilia in induced sputum 24 h after allergen was reduced by 11-fold, and the PC₂₀ methacholine at the same time point was increased by 1.6 doubling doses. These findings provide strong support for a major role for IgE in both early- and late-phase airway responses and acquired hyperresponsiveness in asthma (55).

The clinical efficacy of E25 has been further assessed in moderate-severe allergic asthma in a double-blind, placebo-controlled trial (56). After a run-in period of 4 weeks, in which corticosteroids and β_2 -agonists were adjusted, 317 subjects were randomized to i.v. E25 twice weekly using either a low dose (0.006 mg/kg/IU/ml), a high dose (0.014 mg/kg/IU/ml), or placebo for a period of 12 weeks. Before treatment there were no significant between-group differences in baseline measures with a FEV₁ mean percent predicted of 71% and PEF 380 l/min, symptom score (median 4 on a 7-point scale), inhaled corticosteroid (median 800 μ g/day), oral corticosteroid (median 10 mg/day), and inhaled β_2 -agonist (median 7 puffs/day). Baseline median serum total IgE levels were 619, 564, and 480 ng/ml in the low-dose, high-dose, and placebo groups, respectively. Each of the two active treatments reduced the total serum IgE level by 99%, associated with a 42% improvement in both the high and low active-treatment groups, compared to 23% with placebo. With the dose, the symptomatic response was accompanied by a 31 l/min increase in mean morning PEF and a 20 l/min increase in mean evening PEF. These results provided evidence that 12-week therapy with E25 resulted in improvements both in symptoms and pulmonary function in moderate-severe asthma (56).

By continuing treatment for a further 8 weeks, it was also possible to assess any corticosteroid-sparing effect of E25 (57). Corticosteroid use fell from baseline by 24% after placebo, 41% after low-dose E25, and 50% after high-dose E25. Mean decrease in oral corticosteroid use also favoured E25: 65% decrease for the low dose, 50% for the high dose, and 0% for placebo. Of additional importance, during the course of this study, E25 decreased exacerbations of asthma. Daily total symptom scores and β_2 -agonist usage were also significantly decreased in both active-treatment groups. Further clinical studies have shown that, when administered to atopic subjects with perennial rhinitis for 16 weeks, E25 reduced the skin wheal response to allergens by 60% after the lower dose and 80% after the high dose (57). When administered for 12 weeks to seasonal allergic rhinitis patients, E25 produced highly significant reductions in symptoms and use of escape medication (58). In contrast to systemic administration, local administration of E25 to asthmatic airways, daily for 8 weeks, failed to inhibit either the early or the late response to allergen challenge, indicating that, for efficacy, it is removal of circulating IgE that is required (59). Another possibility is that by the inhaled route, neutralizing antibodies are generated. A recent study (60) has shown that E25 is actively transported across the respiratory epithelium by a mechanism that has not yet been elucidated.

An important finding has been the discovery that treatment with E25 causes downregulation of Fc ϵ R1 expression on human basophils (and presumably mast cells) from a median of ~220 000 to 8300 receptors per cell with a $t_{1/2}$ of 3 days (61). These fascinating findings support the view that Fc ϵ R1 density is directly or indirectly regulated by free IgE. Discontinuation of the E25 infusion results in Fc ϵ R1 expression returning to normal in parallel with restoration of circulating free IgE levels (62).

Concluding comments

These studies strongly indicate an important role for IgE in atopic asthma and rhinitis, and the results provide options for the development of a novel therapeutic agent based on this mechanism. A reduction of IgE levels by 80–90% has been shown to improve symptoms of rhinitis and allergic asthma. However, what is not yet understood is why some patients respond well to this therapy, whereas in others the clinical response is low despite the fact that no immunologic differences between “responders” and “nonresponders” have been identified. Even after 20 weeks of treatment, very few asthmatic patients showed total remission of their disease. The question of whether long

treatment will result in total remission must await longer term trials, but, at the time of writing, there would appear to be a good case for believing that inflammatory mechanisms in asthma extend beyond IgE triggering.

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