

EDITORIAL

Blocking antibodies in allergen immunotherapy: the Yin and Yang

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Specific immunotherapy (SIT) is an established treatment for selected allergic diseases including pollen, bee venom and dust mite allergy. However, despite its proven efficacy, the mechanism of action requires further clarification. Based upon observations from SIT-treated patients, several theories have been proposed. These include (1) induction of T cell tolerance or functional silencing (anergy) [1], (2) an altered balance of cytokines such that there is a decreased abundance of 'allergy-associated' Th2 cytokines relative to 'non-allergic' Th1 cytokines [1–5], (3) increased production of the 'immunosuppressive' cytokines IL-10 and TGF- β [5] by regulatory (T_R , NKT and $CD4^+CD25^+$) T cells [6–9; Gardner et al., submitted], and (4) the induction of IgG 'blocking' antibodies which antagonize the actions of allergen-specific IgE antibody [10, 11].

SIT historically involves administration of relatively crude allergen extracts. However, SIT using natural allergen extracts cannot be used for certain allergies, such as peanut and latex, due to the high risk of life-threatening anaphylaxis. The advent of recombinant DNA technology has permitted production of purified recombinant allergens that offer exciting potential for the tailored treatment of allergies. One approach involves site directed mutagenesis such that IgE-reactive regions (epitopes) are destroyed while retaining immunodominant T cell epitopes [12, 13]. A complementary approach uses short or long T cell peptides (containing single or multiple epitopes, respectively), which do not bind to or cross-link IgE due to disrupted tertiary structure; this concept has shown promise both experimentally and in human trials [14–17].

Although the blocking antibody concept has been established for over 60 years [11], there has been debate regarding the importance of such antibodies. A general observation is that during SIT increases in allergen-specific IgG1 and IgG4, antibody concentrations are related to improvements in clinical outcome [8, 18–20]. Frequently, however, successful SIT is not associated with a decreased concentration of allergen-specific IgE [4, 8, 19, 20], although this is not always the case [11, 21, 22]. The primary mechanisms via which blocking antibodies are thought to exert their effects are via (1) inhibition of basophil histamine release [23, 24], and (2) inhibition of IgE-facilitated allergen presentation by B cells to allergen-specific T cells, an effect that is dependent on the B cell low affinity IgE receptor CD23 [25, 26]. The finding that human monoclonal IgG antibodies specific for different epitopes of the birch pollen allergen Bet v 1 can either inhibit or enhance the binding of IgE from Bet v 1-allergic patients, demonstrates the importance of the antibody fine specificity [23]. Recent data indicating that the efficacy of blocking antibodies is primarily a function of their serum concentration, and is not dependent on the avidity with which they bind allergen [27], suggests that antibody affinity maturation does not play a major mechanistic role.

As mentioned above, disruption of IgE-binding regions of allergens represents a promising approach for SIT. Using genetic engineering, two non-overlapping fragments of the Bet v 1 allergen were generated, which showed negligible binding of IgE from birch pollen-allergic individuals, while retaining T cell-reactive epitopes [28]. Mice immunized with these 'hypoallergenic' fragments produce IgG antibodies, which inhibited the binding of allergic patients' IgE to Bet v 1 [29]. Furthermore, intranasal instillation of the immunodominant Bet v 1 fragment (containing the major T cell epitope) prevented subsequent birch pollen-allergic responses in mice [30]. Using similar logic, Bet v 1 trimer, produced by covalently linking three Bet v 1 molecules, showed diminished ability to induce inflammatory mediator (histamine and leukotriene) release from allergic patients' granulocytes, and induced antibodies that blocked the binding of allergic patients' IgE to Bet v 1 [31].

As a continuation of this series of investigations, and as a prelude to clinical studies, Mahler et al. [32] further characterized their hypoallergenic Bet v 1 constructs. Mice immunized with aluminium hydroxide-adsorbed Bet v 1 fragments and trimer induced relatively weak IgE responses and strong IgG1 responses. The key findings are that (1) IgG1 from mice immunized with Bet v 1 fragments or trimer react with wild-type Bet v 1 (and the related alder and hazel pollen allergens), (2) that these IgG antibodies inhibit the binding of IgE from Bet v 1-allergic humans to wild-type Bet v 1, (3) serum from mice immunized according to the 'high-dose' schedule did not trigger basophil histamine release *in vitro*, and (4) serum from rabbits immunized with hypoallergenic Bet v 1 inhibited mediator release from rat basophil leukaemia cells. The implication of these findings is that clinical treatment with hypoallergenic allergens may induce blocking antibody capable of lessening the severity of allergic reactions to wild-type allergen.

Despite these findings, questions remain regarding the ability of blocking antibodies to alter the course of established allergic disease. Clinically, the relevance of blocking antibodies is disputed, and it is possible that they are induced as side-effect of SIT. In this context it should be noted that several groups have failed to show a correlation between the presence of blocking antibodies and improved clinical outcome [33–35]. The observation that blocking antibodies can inhibit the binding of allergen-specific IgE *in vitro* [26, 27, 29, 31, 32] does not necessarily imply a physiological role; the finding that sera from SIT patients could inhibit allergen-dependent IgE binding to B cells, even when such sera was added *secondarily* to atopic patient serum [26], may be due to a molar excess of allergen-specific IgG relative to IgE antibodies, and establishment of binding equilibrium. Thus an important issue is whether the relative concentrations of

blocking IgG vs. IgE antibodies used *in vitro* reflect the situation *in vivo*. The fact that serum from rabbits immunized with hypoallergenic Bet v 1 mutants inhibits mediator release from rat basophil leukaemia cells [32] and that serum from patients undergoing SIT inhibits basophil histamine release [24] partially addresses the issue of physiological relevance, although formal *in vivo* confirmation is required. Finally, although it has been shown that blocking antibodies can interfere with B cell (CD23)-mediated IgE-dependent allergen presentation [25, 26], the complex biology of CD23 regulation means its physiological role is unresolved [36]. Nevertheless, possible immunotherapeutic approaches include immunization with hypoallergenic allergens tailored to induce blocking antibodies, or direct instillation of allergen-specific blocking antibodies into the airways, although in the latter case serum sickness may be a problem. With regard to the second possibility it is noteworthy that intranasal delivery of allergen-specific IgG to mice inhibits allergic airways inflammation, a process that is dependent on the Th1 cytokine IFN- γ and cell-surface IgG-specific receptors (Fc γ R) [37]. However, another study indicated that the binding of blocking IgG to B cells was not obligatory for the inhibition of IgE binding [26].

A variety of cell types and mechanisms regulate the outcome of an immune response. It is now clear that mature/activated dendritic cells play a central role in both the induction and maintenance of allergic airway disease [38]. Furthermore, accumulating evidence suggests that DC have a relatively 'plastic' differentiation potential, such that they respond to environmental and immunological stimuli. Thus, the observation that DC recognition of microbial products is *necessary* for the generation of allergic (Th2-type) immune responses provides clues regarding potential therapy for allergic diseases [38]. Additionally, it is apparent that a variety of regulatory T cell types (including T_R, NKT and CD4⁺CD25⁺ T cells) play important roles in suppressing asthma and allergy [9]. Furthermore, it is generally accepted that immunological processes occurring during early childhood play a major role in shaping our subsequent susceptibility to allergic disease and asthma [39, 40]. Thus, achieving the dual goals of decreasing asthma and allergy prevalence, and disease severity in affected individuals, will presumably require dietary/environmental and immunotherapeutic interventions. Future studies will need to address whether blocking antibodies exert their effects by modulating key immunological processes such as DC differentiation and regulatory T cell function. A possible approach would be to study the effects of systemically delivered allergen-specific IgG antibodies in murine models of allergy. On a practical level, human therapy would require large-scale production of blocking antibodies and development of simple and effective delivery protocols. Alternately, recombinant/mutant allergens capable of inducing blocking antibody of appropriate specificity may be utilized. Whether or not such blocking antibody therapy could alter the course of established human disease over long periods remains to be seen.

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