

EDITORIAL

Induction of 'blocking' IgG antibodies during immunotherapy

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Specific allergen immunotherapy (SIT) to date is the only treatment shown to have long-term benefit in patients with IgE-mediated allergic disorders such as allergic rhinitis. In seasonal rhinitis and asthma, and to a lesser extent in perennial allergic rhinitis, SIT is highly effective in reducing symptoms and medication use in patients who fail to respond adequately to usual pharmacologic treatments and these effects are maintained for at least three years after discontinuation [1–6]. Clinical outcomes such as early and late skin, nasal and bronchial reactions have been shown to decrease after treatment. For example, immunotherapy results in a reduction in immediate allergen-induced symptoms and the concentrations of inflammatory mediators in nasal lavage fluid, including histamine and prostaglandin D₂ [7]. The risk/benefit of SIT is less favourable in patients with chronic asthma because of an increased risk of developing IgE-mediated systemic side-effects and, rarely, anaphylaxis. Therefore, delineation of the immunological mechanisms underlying successful immunotherapy, with a view to develop more targeted forms of therapy with maintained efficacy but reduced risk of side-effects, remains an important goal.

While the clinical efficacy of SIT is well-documented, the molecular mechanisms are incompletely understood. Current evidence suggests that specific immunotherapy exerts an effect on several aspects of the immune system, including modulation of allergen-specific B cell as well as T cell responses. Studies on the effect of immunotherapy have demonstrated reduced basophil reactivity to allergens [8], reductions in mucosal recruitment of inflammatory cells [9], deviation of Th2 cytokine responses to allergens in favour of Th1 responses [10, 11–13] and the induction of IL-10 producing regulatory T cells [14–16]. In addition, changes in serum antibody titres in response to immunotherapy have been described, mostly as increases in allergen-specific IgG antibodies, particularly the IgG4 isotype [17, 18–22], sometimes accompanied by blunting of seasonal increases in IgE antibodies.

In order to improve safety while maintaining efficacy, attempts have been made to reduce allergen-induced IgE responses during the course of immunotherapy. For example, one therapeutic approach has been the use of novel vaccine adjuvants based on bacterial lipopolysaccharide (LPS). LPS is a bacterial membrane component specific to Gram-negative bacteria, which acts as an immunomodulator by activating the innate immune response. LPS consists of a complex glycolipid composed of a hydrophilic polysaccharide portion and a hydrophobic domain known as lipid A, which contains the adjuvant activity of LPS [23]. The clinical use of LPS is limited because of toxicity, including possible induction of a sepsis-like systemic inflammatory response syndrome. Chemical modifications of LPS have been developed such as a

detoxified version of the lipid A moiety of *Salmonella minnesota* R595 termed monophosphoryl lipid A (MPL). MPL retains immunostimulatory properties without the potential toxic systemic side-effects of LPS [24]. MPL stimulates Th1-type antigen specific responses in rodent models including preferential induction of IgG antibodies while inhibiting IgE [24–27]. These properties make it potentially useful as an adjuvant for allergen-specific immunotherapy.

Studies in mice have shown that co-administration of MPL with a ragweed pollen vaccine results in enhanced IgG_{2b} responses, suggesting a strong Th1-inducing bias as an adjuvant for promoting ragweed-specific antibodies [28]. Moreover, MPL treatment of rats reduced antigen-specific IgE responses upon booster injections in hypersensitized animals. These results suggest that co-application of MPL with allergen vaccine during SIT could result in enhancement of Th1 responses while downregulating allergen-specific IgE responses, thereby improving efficacy and reducing potential side-effects. A controlled trial of grass pollen extract containing an MPL adjuvant was successful in reducing nasal and ocular symptoms and requirement for rescue medication when compared to a placebo group [29]. In addition, there was an increase in allergen-specific IgG antibody titres and a blunting of seasonal increases in allergen-specific IgE in the actively treated group.

Significant increases in specific IgG antibodies may be of clinical importance by preventing IgE-mediated activation of mast cells, which contribute to the early-phase response in allergic inflammation. However, quantitative changes in IgG antibody do not always correlate with clinical protection [18, 19, 30]. For this reason, the role of IgG in immunotherapy has been questioned and it is not clear whether the significant increases in IgG have a causal role in alleviating symptoms or simply represent a bystander effect, occurring as a consequence of high allergen exposure. To refute a role for allergen-specific IgG on the basis of a lack of correlation between clinical response and quantity of antibody is probably too simplistic. Michils et al. [20] investigated the IgG antibody response to venom immunotherapy and observed increases in IgG titres following IT. However, the investigators also reported for the first time that this was preceded by a rapid change in the fine specificity of IgG antibodies. In a more recent study, the same investigators reported that allergen-specific IgG isolated from patients allergic to bee venom displayed a fine specificity spectrum to the major bee venom allergen that was distinct from that of allergen-specific IgG derived from individuals protected either naturally or by successful immunotherapy [31]. These data complement findings from murine models of immunotherapy, where the administration of high concentrations of allergen has been shown to alter antibody affinity and specificity as

well as the quantity of antibodies produced. Kolbe et al. [32] immunized mice with allergen and found that small concentrations of allergen induced IgE and IgG antibodies against distinct and non-overlapping epitopes, whereas at higher allergen concentrations the discrimination disappeared and IgG antibodies were induced that shared epitopes with IgE and therefore had blocking activity. These findings are consistent with the view that immunotherapy may act to change the character of allergen-specific IgG as well as simply increasing concentrations. It is likely that simply measuring serum concentrations of allergen-specific IgG may be too crude a measure to define altered biological reactivity of IgG following IT.

For these reasons, it is necessary to measure both the altered functional as well as immunoreactive IgG activity following immunotherapy in order to define its *in vivo* significance. IgG antibodies exhibit *in vitro* blocking activities on leucocyte histamine release and could also play a role in inhibiting signal transduction and mediator release through the high-affinity IgE receptor, FcεRI, by cross-linking mast cell IgE (FcεRI) and IgG (FcγRIIB) receptors [33–36]. In addition, changes in allergen-specific IgG₄ antibodies induced by immunotherapy have been shown to be associated with blocking allergen-induced IgE-dependent histamine release by basophils [37].

In the present issue of this journal, Mothes et al. [38] present data from a randomized, double-blind, placebo-controlled multi-centre study using an MPL-adjuvanted grass pollen vaccine. In addition to measuring clinical improvement and changes in allergen-specific IgE, IgG and IgM antibody titres using recombinant Phl p 1, Phl p 2 and Phl p 5, the authors also assess functional changes in IgG antibody titres by measuring inhibition of allergen-specific IgE-mediated histamine release by basophils. The authors report a strong induction of Phl p 5-specific IgG₁ and IgG₄ responses and a reduction in seasonal increase in allergen-specific IgE, which correlated with clinical improvement. Moreover, changes in antibody IgG titre were also associated with induction of a functional blocking activity as measured by inhibition of allergen-dependent basophil histamine release. These results provide further evidence that inhibition of IgE-mediated histamine release by mast cells and basophils upon cross-linking with allergen by IgG antibodies is likely to play a role in reducing the early-phase response of an allergic reaction. Moreover, they suggest a causal link between induction of allergen-specific IgG responses, improvement in immediate-type responses to allergen and 'functional' IgG-blocking activity. The findings by Mothes et al. suggest that MPL-adjuvanted grass pollen allergy may be effective, at least in part, by induction of IgG antibodies with the capacity to block IgE-mediated mechanisms. The contributory role of MPL in the development of these changes remains to be assessed since conventional SIT without adjuvant has previously been reported to induce similar changes.

In addition, the role of MPL on T cell responses in SIT where MPL is employed as an adjuvant remains to be addressed. While administration of ragweed allergen covalently linked with immunostimulatory sequence (ISS) DNA derived from bacteria has been shown to enhance IFN-γ production in peripheral blood mononuclear cell cultures from treated patients [39], it is now clear that Th1-skewing

adjuvants may inhibit allergic inflammation by mechanisms independent of IFN-γ and IL-12. Moreover, induction of proinflammatory cytokines such as IFN-γ could potentially exacerbate inflammation. In this context, induction of regulatory cytokines, such as IL-10, as opposed to up-regulation of Th1 responses, might be a more appropriate goal for treating allergic disease. In addition to its inhibitory effects on both Th1 and Th2 cytokines, a variety of other immunologic properties make this cytokine an important target for immunotherapy of allergic disease. These include inhibition of activation of human lung mast cells and selective enhancement of IgG₄ with suppression of IgE [40, 41]. Studies on bee venom and grass pollen IT have reported an induction of IL-10 in response to treatment [14–16], and more recently, increased expression of IL-10 mRNA in the upper respiratory tract and recruitment of T cells to the skin has also been reported in patients receiving allergen-specific grass pollen IT [42, 43]. Moreover, certain bacterial adjuvants such as CpG ODN have been shown to induce IL-10 as well as IFN-α in cells from atopic patients [44]. It remains to be established if MPL also has an effect on IL-10 induction when used as an adjuvant in SIT. The increases in allergen-specific IgG₄ titres with no significant changes in IgG₂, as reported by Mothes et al., strongly suggest that MPL-linked SIT may induce a regulatory response rather than a Th1 response. However, further studies are needed to address whether B cells or T cells are the primary targets for MPL-adjuvanted SIT.

The data from Mothes et al. suggest that MPL immunotherapy may induce changes in IgG antibodies after only four preseasonal injections. To date, relatively few studies have investigated the time course of IgG induction during conventional immunotherapy. Studies in bee venom IT suggest that changes in antibody specificity pre-empt changes in antibody titres and it remains to be assessed whether this holds true for IT with other allergens. The study also emphasizes the value of measuring functional blocking activity of IgG antibodies, which seem to be more closely correlated to clinical outcome than simply indicating alterations in immunoreactive serum IgG.

IgE has important additional biologic properties as well as allergen-IgE dependent triggering of mast cells and basophils. Evidence suggests that IgE is able to bind antigen and focus it to the surface of B cells via binding to CD23. In particular, specific IgE antibodies are able to capture allergen at low concentrations and facilitate presentation via binding to the low-affinity (CD23) or high-affinity receptor (FcεRI) on antigen-presenting cells [45–47]. Antigen-specific IgE bound to CD23 or FcεRI allows a 100-fold reduction in the concentration of antigen required for effective antigen presentation to antigen-specific T cells [46, 48, 49]. Together with the observation that CD23, FcεRI and IgE are increased on cells from patients with allergic disease, these data suggest that IgE-facilitated allergen presentation could also play a role in the presentation of low allergen concentrations to allergen-specific T cells. Indeed, van der Heijden et al. [45] provided the first evidence that elevated serum-IgE levels in house dust mite-allergic patients were able to increase antigen presentation by Epstein–Barr (EBV)-transformed B cells to a T cell clone *in vitro*. This IgE-mediated facilitated allergen presentation (FAP) may play an important role in augment-

ing T cell responses to environmental allergens, with enhanced proliferative and cytokine responses. In the context of mucosal allergy, this mechanism may augment and/or enhance both sensitization and establish Th2 responses to environmental allergens.

In this context, IgG antibodies may have potential blocking activity for IgE-mediated allergen presentation. In contrast to IgE, IgG antibodies, although capable of binding the allergen, do not seem to support facilitated allergen presentation and their presence in sera from immunotherapy-treated patients has been shown to inhibit IgE-mediated facilitated allergen presentation *in vitro*. In a cross-sectional study on a small number of birch pollen immunotherapy-treated patients, van Neerven et al. [17] reported that immunotherapy-induced serum IgG antibodies were able to reduce CD23-dependent IgE-facilitated allergen presentation by B cells to T cell clones at low allergen concentrations. More recently, using a novel flow cytometric assay that measures binding of allergen-IgE complexes to CD23 on EBV-transformed B cells, we could show that increases in Phl p 5-specific IgG antibody titres in sera from immunotherapy-treated patients collected during a placebo-controlled, double-blind trial of grass pollen immunotherapy were associated with inhibition of allergen-IgE binding [22]. Furthermore, by purifying IgG from sera of immunotherapy-treated patients we confirmed that the inhibitory activity was contained in the IgG fraction. Moreover, we found that inhibitory activity was associated with improvement in clinical symptoms after treatment.

Together with the results from Mothes et al. in the present issue of the journal, these findings confirm that immunotherapy induces quantitative and qualitative changes in antibody responses that may have protective effects by inhibiting immediate-type reactions by reducing cross-linking of IgE on mast cells and basophils, and also on the late-phase response by inhibiting IgE-mediated facilitated allergen presentation of B cells to T cells. Assessment of the functional consequences of immunotherapy-induced increases in IgG antibodies, as determined by their blocking ability on IgE-mediated mechanisms such as basophil histamine release and facilitated allergen presentation, could provide a better approach for studying the possible predictive value of IgG responses for successful immunotherapy.

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