

ORIGINAL ARTICLE

Mechanisms of immunotherapy and surrogate markers

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Abstract

Understanding mechanisms of inducible antigen-specific tolerance will improve immunotherapy and reveal the most relevant biomarkers for objectively measuring response to immunotherapy in clinical trials and routine practice. Various physiological and laboratory parameters are proposed as biomarkers of an immunological response to vaccines, although their surrogacy for clinical end points is unproven. Examples of physiological biomarkers include suppression of allergen-induced early- and late-phase responses. Laboratory biomarkers include increases in bioactive allergen-specific IgG and IgA levels and T-cell interleukin (IL)-10 production. There is a continuing unmet need for biomarkers that will allow prediction of treatment efficacy and assist in monitoring the response after starting therapy or postwithdrawal.

The allergic response is characterized by dysfunction of the allergen-specific T-cell repertoire. This involves an expansion of allergen-specific T cells compared with the natural nonallergic state, together with a shift in favor of pathogenic effector T-helper (Th)2 cells and possibly the emergence of other pathogenic Th effector populations. Downstream consequences include an IgE response with numerous proinflammatory effects including at the level of allergen-presenting cells (APCs). For example, IgE bound to both high- and low-affinity receptors on dendritic cells and other APCs takes up small quantities of allergen, priming the cells for presentation to T cells and contributing to expansion and activation of the allergen-specific T-cell population.

In immunotherapy, the T-cell response is recalibrated, restoring the functional predominance of regulatory T cells. Diminution of the Th2 effector cells occurs over time, with a subtle shift in favor of nonpathogenic Th1 interferon- γ -producing cells. The most important mediators of tolerance in immunotherapy are probably IL-10 and transforming growth factor (TGF)- β . In the context of IL-4-rich responses, IL-10 may trigger production of IgG₄. Signals driving this induction are less well defined for IgA. A consequence of these non-IgE allergen-specific antibodies is antagonism of IgE responses at the level of mast cell and basophil activation,

and competition with IgE for allergen binding on APCs. Over the medium to longer term, this may lead to contraction of the allergen-specific T-cell repertoire, possibly resulting from inhibition of IgE antigen presentation. IL-10 and TGF- β may also directly inhibit T-cell activation and expansion.

Cellular responses to immunotherapy

A recent grass pollen subcutaneous immunotherapy (SCIT) study (1) had an up dosing period of 8–10 weeks, followed by maintenance injections for the 12-month study period. Peripheral blood T cells stimulated with antigen showed the appearance of an IL-10 signal from T cells at 2 weeks after first vaccine exposure, which was statistically significant at 4 weeks. Another study used allergen-specific tetramers in a novel way to investigate antigen-specific T cells during wasp venom SCIT (2). The results challenge the hypothesis that there is a rapid decline in allergen-specific T-cell numbers. T cells examined with tetramers directly *ex vivo* showed a priming effect in the first 4 weeks with transient expansion of cell numbers, which then declined to pretreatment baseline levels over time. Intracellular expression of the FOXP3 transcription factor, thought to be a marker of T-regulatory cells, was

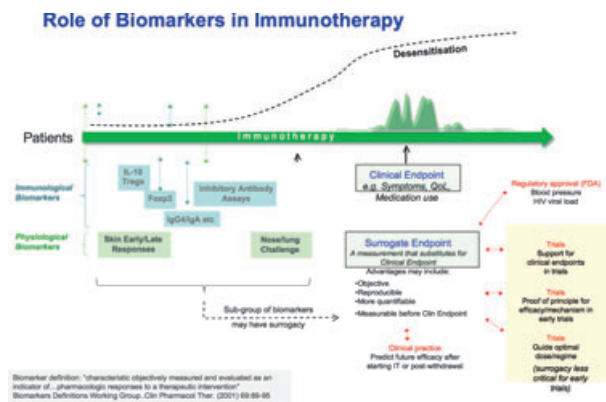


Figure 1 Role of biomarkers in immunotherapy.

increased in these tetramer+ allergen-specific T cells. Induction of IL-10 was also seen, together with the emergence of a Th1 response at the expense of the Th2 response over a 3-month period.

Our knowledge of peripheral T-cell responses in sublingual immunotherapy (SLIT) is less extensive. There are relatively small number of studies in the literature, but perhaps there are also negative studies that have not yet been published. Nonetheless, two reports are especially informative. In one, T-cell proliferation to the major Bet v 1 allergen was measured in patients receiving birch pollen SLIT (3). As early as 4 weeks into treatment, proliferation was reduced, and this was reversed by neutralizing IL-10 or depleting CD25+ T cells. These data suggest that at least early during SLIT, IL-10-producing regulatory T cells may be present systemically. Interestingly, an irrelevant antigen (tetanus toxoid) also showed IL-10-dependent suppression at 4 weeks that later disappeared, perhaps also indicating nonantigen-specific effects. A house dust mite SLIT study (4) found reductions in proliferation in response to treatment, particularly at the 6-month time point. These reductions were partially reversed by antagonising TGF- β . This response was superimposed on the emergence of FOXP3+ T cells, although the allergen specificity of these cells is unknown. Thus, the limited literature on SLIT broadly supports the emergence of systemic regulatory T cells. However, the data are less clear-cut than for SCIT, and perhaps, immunomodulation in SLIT is more localized to mucosal tissues.

Antibody responses to immunotherapy

Our grass pollen time course study of 12 patients (1) found sustained IgG₄ induction over the first few months, significant at 8–10 weeks but with a signal starting at 4–6 weeks. IgA and IgG₁ antibodies appeared to track the IgG₄ response. IgE showed a transient induction in the first few weeks of immunotherapy, which later dropped to pretreatment levels. This is consistent with other studies, so the clinical effects of immunotherapy are not currently explainable by effects on allergen-specific IgE levels.

Antibody functions were evaluated in two bioassays using sera from immunotherapy patients. One assay was dependent on the high-affinity IgE receptor pathway and measured histamine release from basophils of grass pollen allergenic donors. The other looked at the formation of IgE-allergen complexes that bound low-affinity receptors on B cells. Both showed a marked increase in inhibitor activity at 4–6 weeks. The IgG₄ response was somewhat different, suggesting that a functional assay may be more relevant for measuring immunotherapy responses.

In collaboration with ALK, we developed a facilitated allergen-binding (FAB) assay for analyzing functional inhibitory serum antibodies. IgE is incubated with allergen to form complexes that bind to low-affinity IgE receptors on B cells. IgE not complexed with allergen binds B cells only in small quantities at low-affinity receptors. However, IgE-allergen complex formation creates a multivalent entity that binds to low-affinity IgE receptors with high avidity. The complexes can be detected on B cells by flow cytometry with fluorescein isothiocyanate-tagged anti-IgE. When immunotherapy sera are used, complexes do not form because serum non-IgE antibodies antagonise IgE-antibody binding. We tested this assay in several clinical settings to determine whether it provides different information than absolute antibody titer measurements. In one study, we examined antibodies from patients in a large clinical trial of grass pollen SCIT (5, 6) in which two active treatment arms received different maintenance doses. The treatment was effective, and IgE-FAB assay results correlated with combined symptom and medication scores, whereas IgG₄ levels did not. This correlation was significant, although not sufficiently so to recommend use of the assay to predict treatment responses in individual patients. Nonetheless, the results support our hypothesis that bioassays for antibodies are more likely to yield useful information about immunotherapy response than absolute antibody levels.

IgG₄ levels in SLIT and SCIT can be compared indirectly in different clinical studies. For example, when a SLIT group received a grass pollen extract, in liquid form, with a daily maintenance dose of 75 000 SQ-U, a three to fourfold increase in IgG₄ was observed (7). A SCIT group receiving the conventional SCIT maintenance dose of 100 000 SQ-U showed a 30–40-fold increase in specific IgG₄ (8). However, SCIT is a systemic treatment measured as a systemic antibody response, while SLIT is a locally applied oral vaccine, so perhaps systemic antibodies are a less optimal measurement for this treatment.

Regarding long-term effects, patients who receive SCIT for grass pollen for two years show reversal of induced IgG₄. However, the levels do not quite return to baseline (7), even 3 years after discontinuation. These patients remain in clinical remission so we measured the function of the small amount of remaining specific antibody that persists. This had high blocking-antibody activity in the IgE-FAB assay, indicating discordance between antibody levels and *in vitro* function. Future work is directed to characterizing the antibody populations that survive discontinuation.

Progress in biomarker development

Using grass pollen as a model, we identified a number of immune parameters that can change early, even before symptoms can be measured during the pollen season. We also demonstrated physiological changes based on allergen challenge to demonstrate changes early in treatment. A biomarker is something that can be objectively measured and that changes in response to an intervention (9). However, an important question is whether any of these biomarkers have surrogacy and can substitute for true clinical end points like symptom scores. A useful biomarker will measure events before the true clinical end points are apparent and will therefore predict treatment response. Biomarkers may also offer practical advantages over measuring true clinical end points, for example being more objective, quantitative, or reproducible. However, for current immunological markers,

surrogacy evidence is lacking or data indicate poor surrogacy. For regulatory approval purposes, tight correlation between biomarkers and clinical end points would be critical, and some biomarkers currently meet this standard in other areas of medicine. Nonetheless, biomarkers without proven surrogacy can have value in clinical studies, particularly early translational studies of novel therapeutics and in defining treatment protocols with the goal of establishing proof of concept or proof of mechanism prior to large, definitive clinical trials.

Conflict of interest

Stephen Till has received consultancy fees from Glaxo-SmithKline and lecture fees from GlaxoSmithKline and ALK-Abelló.

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