

Chapter 9

Surrogate Markers for Allergen-Specific Immunotherapy

Jiu-Yao Wang

Abstract Allergen-specific Immunotherapy (AIT) still remains the only option for the long-term management of allergic rhinitis and allergic asthma, including disease due to house dust mite (HDM) allergy. In vivo provocation tests (e.g., skin testing and conjunctival provocation testing) are useful indicators of clinical improvement, but the identification of in vitro markers for monitoring the effects of AIT has been a long-sought goal. Previous studies on AIT in IgE-mediated allergic diseases found that suppression of allergen-induced late skin responses occurred as early as 2–4 weeks and was accompanied by increases in IL-10, whereas suppression of the immediate skin response to allergen occurred later, at 6–12 weeks, and was accompanied by increases in serum ‘blocking’ IgG4 antibodies that had the capacity to suppress both allergen-triggered basophil histamine release and the binding of IgE-allergen complexes to B cells. It has been claimed that a flow cytometry-based assay (IgE-FAB) can be used to demonstrate IgE-facilitated antigen presentation and activation of T cells. Measurement of peripheral T cell responses is complex and difficult to standardize for routine clinical use. Whether quantitative measures of IgG-associated inhibition (in contrast to IgG levels) and/or suppression of early and/or late skin responses may predict AIT efficacy and long-term benefits in individual patients remains to be determined. The search for adequate biological markers that reflect the immune status of patients before, during, and after AIT is critical to evaluate its clinical efficacy and the future of personalized medicine in treating allergic diseases.

Keywords Allergen · Immunotherapy · Biomarkers · IgG4 blocking antibody · Basophils activation tests

J.-Y. Wang (✉)

Department of Pediatrics, Allergy and Clinical Immunology Research (ACIR) Center,
College of Medicine, National Cheng Kung University, No.1, University Road,
Tainan 70428, Taiwan
e-mail: a122@mail.ncku.edu.tw

9.1 Introduction

Allergen immunotherapy is highly effective in selected patients with IgE-mediated allergic rhinitis and mild asthma including those who fail to respond to usual anti-allergic drugs. Clinical efficacy may be accompanied by disease remission following discontinuation of treatment and prevention of new sensitizations and/or progression from rhinitis to asthma (Passalacqua and Durham 2007). These clinical observations imply long-term effects on the immune system with induction of immunological tolerance. Data from studies of peripheral blood and target organs suggest that induction of regulatory T cells and/or deviation from antigen-specific T_H2 in favor of T_H1 responses are relevant. Serum measurements imply additional altered long-term memory B cell responses, including increases in allergen-specific IgG, particularly IgG4 and IgA, especially, the IgA2 subclass. Recent studies of the mechanisms of sublingual immunotherapy have identified more modest but similar changes compared to the subcutaneous route (Lee et al. 2013; Moingeon et al. 2006; Scadding and Durham 2009). A critical question is whether these studies may provide biomarkers that may be indicative and/or predictive of the clinical response to immunotherapy.

9.2 The Need for Biomarkers

Biological markers are objectively measured indicators of normal biological processes, pathogenic processes, or pharmacologic responses to therapeutic intervention (Group 2001). Currently, they are of major interest for patient management in future approaches to treatment in all domains of immunotherapy (e.g., allergy, cancer, autoimmunity). For the diagnosis and treatment for allergic diseases, biomarkers should allow allergists to accomplish two things: (1) to identify, prior to immunotherapy, responder versus nonresponder patients with a high degree of confidence and (2) to follow the immunological status of patients during immunotherapy in order to modulate and adjust treatment schemes. Therefore, biomarkers can be classified into three main types: those able to (1) predict a response to treatment and safety, (2) document short-term efficacy, and (3) monitor long-term efficacy. Each type of biomarker is valuable in the support of patient management, before, during, and after immunotherapy. Whereas significant efforts have been made to identify such surrogates in allergen-specific immunotherapy, documenting the impact on symptom and medication scores in the context of double-blind, placebo-controlled clinical trials remains the only true demonstration of clinical efficacy (Prentice 1989).

9.3 Current Status of Biomarkers in Allergic Diseases

Currently, the diagnosis of allergy relies upon skin prick tests and measurement of allergen-specific IgE reactivity using *in vitro* diagnostic assays such as ELISA (CAP system, Vidas) or microfluidic assay (BioIC) (Shyur et al. 2010). Although these diagnostic methods can define a pattern of sensitization for any given patient, and in some cases precisely identify the allergens involved, they are not appropriate for following changes in the immune status of the patient during treatment. Several markers have been proposed to monitor allergic inflammation (e.g., eosinophil counts or ECP levels in sputum, tryptase in nasal fluid). Another indirect and noninvasive biological measure of airway hyperreactivity (AHR) is the NO content in exhaled air. All of these markers relate to inflammatory symptoms associated with late immune effector mechanisms and as such are likely to be relevant when following the impact of symptomatic treatments such as corticosteroids or anti-histamine. However, allergen-specific immunotherapy (AIT) is a causal treatment that corrects the immune imbalance in allergic patients (from a T_H2 to T_H1 or Treg response) in an allergen-specific manner (Akdis and Blaser 2000; Akdis et al. 2004). Therefore, a biomarker for AIT would be one relevant to the modification of an immune parameter that is involved in early inflammation. Classically, only a limited number of immunological parameters have been monitored in the course of allergy immunotherapy studies and were essentially restricted to measuring modulation of the ratio between IgE/IgG4 titers in the serum. More recently, a potential blocking activity for allergen-specific antibodies has been assessed (Francis et al. 2008). It was shown that allergen-specific blocking IgG antibodies inhibit allergen-induced mast cell and basophil degranulation as well as IgE-facilitated allergen presentation to T cells and is associated with a reduction of *in vivo* sensitivity (Dreborg et al. 2012; Niederberger et al. 2004; Pauli et al. 2008; Shamji and Durham 2011). In addition, measurement of IgE-mediated activation of basophils has been performed (looking either at histamine release or at the expression of the activation marker CD203c). Cellular assays (e.g., basophil activation assays and FAB assay) (Lichtenstein et al. 1973; Shamji et al. 2006) may allow for the uncovering and measurement of the effects of allergen-specific blocking antibodies that interfere with allergen-IgE interaction as well as for the correlation of *in vitro* results with clinical outcomes but they are quite cumbersome. Presently, reduction in allergen-specific IgE binding can be measured by microarray, which may become a possible surrogate marker for the effects of specific immunotherapy (Skrindo et al. 2015). Several studies identified shifts from T_H2 to T_H1 and/or regulatory T cell responses by analyzing PBMC. These latter findings, however, were not reproduced in other studies and, as of today, none of those parameters has been confirmed to correlate with clinical efficacy. They have only demonstrated the establishment of an immune response induced by the vaccine. Thus, the search for adequate biological markers that reflect the immune status of patients and clinical efficacy is critical to support the use of allergen-specific immunotherapy.

9.4 Candidate Biomarkers in Clinical Trials for Allergen-Specific Immunotherapy

Immunotherapy still remains the only option for the long-term management of AR and allergic asthma, including disease due to HDM allergy. AIT is currently administered via the subcutaneous (SCIT) and sublingual (SLIT) routes, which have been shown to be effective and induce long-term clinical and immunologic tolerance. (Durham et al. 1999, 2012; Francis et al. 2008; James et al. 2011). Unlike other pharmacological treatment options, immunotherapy acts on symptoms as well as altering the natural course of the disease. A search for biomarkers can ideally be conducted in allergic patients undergoing immunotherapy. In this context, a rational approach is to collect samples during clinical studies or to set up dedicated clinical studies to assess immunological responses (both humoral and cellular) induced during immunotherapy in association with clinical efficacy. Immunologic changes after immunotherapy include suppression of allergen-specific T_H2 responses, induction of regulatory T cells (IL-10⁺ CD4⁺ CD25hi, and CD4⁺ CD25hi forkhead box protein 3, Foxp3,—positive cells) and the appearance of “protective” allergen-specific IgG antibodies, particularly of the IgG4 subclass (Akdis et al. 1998; Francis et al. 2003; James et al. 2011; Shamji et al. 2012; van Neerven et al. 1999). Suppression of the cutaneous early allergic response after immunotherapy is temporarily associated with increases in serum IgG-associated inhibitory activity but the detailed mechanism of AIT remains elusive.

9.5 Comparison of Immune Responses to Allergen Between Atopic and Healthy Individuals

An additional approach to identify and validate biomarkers is to analyze potential protective responses observed in healthy individual exposed to allergens. Although it was previously thought that nonallergic patients are anergic to allergens from the environment, there is now evidence that they indeed mount allergen-specific responses preventing allergic symptoms. For example, the IgE and IgG reactivity profiles to HDM allergens, as well as IgE levels to certain allergen components, differed considerably between children with and without asthmatic symptoms caused by HDM allergy (Resch et al. 2015). In fact, asthmatic children were characterized by an expanded IgE repertoire regarding the numbers of recognized allergen components and by increased specific IgE levels. To analyze and compare allergen-specific T cell responses in healthy and allergic patients, allergen-specific HLA class II tetramers have been developed. In this approach, T cell epitopes are identified within the allergen, and the major immunodominant epitope is combined with multimeric recombinant HLA molecules labelled with a fluorescent dye. It has been reported that such a reagent was used as a probe to detect and characterize birch pollen and house dust mite-specific CD4⁺ T lymphocytes in the

blood of healthy people (Van Overtvelt et al. 2008). The detailed characterization (e.g. TCR, V beta usage, cytokine profile, transcriptome) of allergen reactive T cells detected in healthy donors or allergic patients using such tetramers could lead to the identification of immunological biomarkers that correlate with protection.

9.6 Biomarkers for Allergen-Specific Immunotherapy? An Unmet Need

In the future, when patients receive allergen-specific immunotherapy, biomarkers should be quantitative and easily measured in readily accessible body fluids (e.g., blood, saliva, nasal secretions) (Horak et al. 2009). Serologic parameters (e.g., antibody titers) can easily be monitored even in the context of large-scale clinical studies because they usually require small volumes of blood and simple logistics. The functionality of these antibodies can further be assessed by measuring their affinity and their blocking potential. Such analyses can be extended to various isotypes possibly involved in immunotherapy efficacy. Exploratory technologies, like proteomic approaches, can also be used to identify new markers modulated during immunotherapy. In contrast, cellular responses can only be assessed in small-scale clinical studies due to the complexity of T cell analysis using fresh blood. There are several assays that can be used to monitor allergen-specific T cells in peripheral blood (e.g., proliferation of CFSE-labeled cells, intracellular cytokine production, ELISPOT, gene expression by real-time PCR) (Keilholz et al. 2002). These assays allow discriminating between allergen-specific T_H1 , T_H2 , and Treg responses. In addition, the development of new tools such as tetramers now permit the detection of allergen-specific cells and may enable better identification of changes during treatment.

9.7 Conclusion

The benefits conveyed by the discovery of biomarkers in the field of immunotherapy for common allergies are numerous. Particularly, biomarkers would be extremely valuable in (1) identifying patients likely to benefit from the treatment (i.e., establishing their immune status before the treatment), (2) adapting treatment modalities such as immunization schemes and dosing to the immune status of the patient (“personalized medicine”), (3) providing tools for immunomonitoring during and after the treatment, (4) establishing biological mechanisms of action of successful immunotherapy, and (5) supporting the approval process for a drug or treatment. We currently lack quantitative biomarkers that are able to reveal the clinical efficacy of allergen-specific immunotherapy. The development of methods to monitor allergen-specific immune responses (or biological changes reflecting

the early consequences of immune stimulation with the allergen) should provide immunomonitoring tools that would be extremely useful to allergists in order to assess the immune status of patients before, during, and after immunotherapy. Ultimately, such tools would be routinely used to adjust the dose and immunization regimen to the needs of the patient. In the absence of such established biomarkers, the clinical efficacy of AIT can only be established in the context of large scale, placebo-controlled clinical studies.

References

- Akdis CA, Blaser K. Mechanisms of allergen-specific immunotherapy. *Allergy*. 2000;55(6):522–30.
- Akdis CA, Blesken T, Akdis M, Wuthrich B, Blaser K. Role of interleukin 10 in specific immunotherapy. *J Clin Invest*. 1998;102(1):98–106.
- Akdis M, Verhagen J, Taylor A, Karamloo F, Karagiannidis C, Crameri R, Thunberg S, Deniz G, Valenta R, Fiebig H, et al. Immune responses in healthy and allergic individuals are characterized by a fine balance between allergen-specific T regulatory 1 and T helper 2 cells. *J Exp Med*. 2004;199(11):1567–75.
- Dreborg S, Lee TH, Kay AB, Durham SR. Immunotherapy is allergen-specific: a double-blind trial of mite or timothy extract in mite and grass dual-allergic patients. *Int Arch Allergy Immunol*. 2012;158(1):63–70.
- Durham SR, Walker SM, Varga EM, Jacobson MR, O'Brien F, Noble W, Till SJ, Hamid QA, Nouri-Aria KT. Long-term clinical efficacy of grass-pollen immunotherapy. *N Engl J Med*. 1999;341(7):468–75.
- Durham SR, Emminger W, Kapp A, de Monchy JG, Rak S, Scadding GK, Wurtzen PA, Andersen JS, Tholstrup B, Riis B, et al. SQ-standardized sublingual grass immunotherapy: confirmation of disease modification 2 years after 3 years of treatment in a randomized trial. *J Allergy Clin Immunol*. 2012;129(3):717–25.
- Francis JN, Till SJ, Durham SR. Induction of IL-10⁺CD4⁺CD25⁺ T cells by grass pollen immunotherapy. *J Allergy Clin Immunol*. 2003;111(6):1255–61.
- Francis JN, James LK, Paraskevopoulos G, Wong C, Calderon MA, Durham SR, Till SJ. Grass pollen immunotherapy: IL-10 induction and suppression of late responses precedes IgG4 inhibitory antibody activity. *J Allergy Clin Immunol*. 2008;121(5):1120–5.
- Group BDW. Biomarkers and surrogate endpoints: preferred definitions and conceptual framework. *Clin Pharmacol Ther*. 2001;69(3):89–95.
- Horak F, Ziegelmayer P, Ziegelmayer R, Lemell P, Devillier P, Montagut A, Melac M, Galvain S, Jean-Alphonse S, Van Overtvelt L, et al. Early onset of action of a 5-grass-pollen 300-IR sublingual immunotherapy tablet evaluated in an allergen challenge chamber. *J Allergy Clin Immunol*. 2009;124(3):471–7.
- James LK, Shamji MH, Walker SM, Wilson DR, Wachholz PA, Francis JN, Jacobson MR, Kimber I, Till SJ, Durham SR. Long-term tolerance after allergen immunotherapy is accompanied by selective persistence of blocking antibodies. *J Allergy Clin Immunol*. 2011;127(2):509–16.
- Keilholz U, Weber J, Finke JH, Gabrilovich DI, Kast WM, Disis ML, Kirkwood JM, Scheibenbogen C, Schlom J, Maino VC, et al. Immunologic monitoring of cancer vaccine therapy: results of a workshop sponsored by the Society for Biological Therapy. *J Immunother*. 2002;25(2):97–138.
- Lee M, Lee BW, Vichyanond P, Wang JY, Bever HV. Sublingual immunotherapy for house dust mite allergy in Southeast Asian children. *Asian Pac J Allergy Immunol*. 2013;31(3):190–7.

- Lichtenstein LM, Ishizaka K, Norman PS, Sobotka AK, Hill BM. IgE antibody measurements in ragweed hay fever. Relationship to clinical severity and the results of immunotherapy. *J Clin Invest.* 1973;52(2):472–82.
- Moingeon P, Batard T, Fadel R, Frati F, Sieber J, Van Overtvelt L. Immune mechanisms of allergen-specific sublingual immunotherapy. *Allergy.* 2006;61(2):151–65.
- Niederberger V, Horak F, Vrtala S, Spitzauer S, Krauth MT, Valent P, Reisinger J, Pelzmann M, Hayek B, Kronqvist M, et al. Vaccination with genetically engineered allergens prevents progression of allergic disease. *Proc Natl Acad Sci U S A.* 2004;101(Suppl 2):14677–82.
- Passalacqua G, Durham SR. Allergic rhinitis and its impact on asthma update: allergen immunotherapy. *J Allergy Clin Immunol.* 2007;119(4):881–91.
- Pauli G, Larsen TH, Rak S, Horak F, Pastorello E, Valenta R, Purohit A, Arvidsson M, Kavina A, Schroeder JW, et al. Efficacy of recombinant birch pollen vaccine for the treatment of birch-allergic rhinoconjunctivitis. *J Allergy Clin Immunol.* 2008;122(5):951–60.
- Prentice RL. Surrogate endpoints in clinical trials: definition and operational criteria. *Stat Med.* 1989;8(4):431–40.
- Resch Y, Michel S, Kabesch M, Lupinek C, Valenta R, and Vrtala S. Different IgE recognition of mite allergen components in asthmatic and nonasthmatic children. *J Allergy Clin Immunol.* 2015.
- Scadding G, Durham S. Mechanisms of sublingual immunotherapy. *J Asthma.* 2009;46(4):322–34.
- Shamji MH, Durham SR. Mechanisms of immunotherapy to aeroallergens. *Clin Exp Allergy.* 2011;41(9):1235–46.
- Shamji MH, Wilcock LK, Wachholz PA, Dearman RJ, Kimber I, Wurtzen PA, Larche M, Durham SR, Francis JN. The IgE-facilitated allergen binding (FAB) assay: validation of a novel flow-cytometric based method for the detection of inhibitory antibody responses. *J Immunol Methods.* 2006;317(1–2):71–9.
- Shamji MH, Ljorring C, Francis JN, Calderon MA, Larche M, Kimber I, Frew AJ, Ipsen H, Lund K, Wurtzen PA, et al. Functional rather than immunoreactive levels of IgG4 correlate closely with clinical response to grass pollen immunotherapy. *Allergy.* 2012;67(2):217–26.
- Shyur SD, Jan RL, Webster JR, Chang P, Lu YJ, Wang JY. Determination of multiple allergen-specific IgE by microfluidic immunoassay cartridge in clinical settings. *Pediatr Allergy Immunol.* 2010;21(4 Pt 1):623–33.
- Skrindo I, Lupinek C, Valenta R, Hovland V, Pahr S, Baar A, Carlsen KH, Mowinckel P, Wickman M, Melen E, et al. The use of the MeDALL-chip to assess IgE sensitization: a new diagnostic tool for allergic disease? *Pediatr Allergy Immunol.* 2015;26(3):239–46.
- van Neerven RJ, Wikborg T, Lund G, Jacobsen B, Brinch-Nielsen A, Arnved J, Ipsen H. Blocking antibodies induced by specific allergy vaccination prevent the activation of CD4+ T cells by inhibiting serum-IgE-facilitated allergen presentation. *J Immunol.* 1999;163(5):2944–52.
- Van Overtvelt L, Wambre E, Maillere B, von Hofe E, Louise A, Balazuc AM, Bohle B, Ebo D, Leboulleire C, Garcia G, et al. Assessment of Bet v 1-specific CD4+ T cell responses in allergic and nonallergic individuals using MHC class II peptide tetramers. *J Immunol.* 2008;180(7):4514–22.

Author Biography



Prof. Dr. Jiu-Yao Wang PhD is currently a Distinguished Professor of Pediatrics and Director of the Allergy and Clinical Immunology Research (ACIR) Center at National Cheng Kung University Medical Center, Tainan, Taiwan. After clinical residency and sub-specialty training in pediatric allergy, clinical immunology and rheumatology from 1984-1987 at the National Taiwan University Hospital, Taipei, he was appointed as Lecturer (1989), Associate Professor (1992), and Professor (2000) at the College of Medicine, National Cheng Kung University, Tainan, Taiwan. He obtained his Doctor of Philosophy (D.Phil.) degree from the MRC Immunochemistry Unit, University of Oxford, United Kingdom in 1996. For more than 25 years, Dr. Wang's lab has been focused on the study of the genetic, environmental, and immunological factors involved in the innate immunity of allergic and asthmatic children

in Taiwan. These studies have produced more than 110 SCI journal papers that have been cited more than 2,000 times. He has received several academic awards, including the 37th Ten Outstanding Young Persons in Medical Research Award (1999) sponsored by Taiwan Junior Commercial Chamber, Taiwan, and Gold Medal of K.T. Li Foundation for the Development of Science and Technology (2014). He has been the president of the Taiwan Academy of Pediatric Allergy, Asthma, and Clinical Immunology (TAPAACI), Board Member and Congress Chair of the 2013 Asian Pacific Academy of Allergy, Asthma and Clinical Immunology (APAAACI), and currently serves as a National Delegate and Board Member of the Educational Council of the World Allergy Organization (WAO).