



Review

Evolution in immunological methods used in research and in the clinical diagnosis and management of human allergic diseases

Robert G. Hamilton *

Allergy and Clinical Immunology Division, Department of Medicine, Johns Hopkins University School of Medicine, Baltimore, MD 21224, USA
Department of Pathology, Johns Hopkins University School of Medicine, Baltimore, MD 21224, USA

ARTICLE INFO

Article history:

Received 14 June 2012

Received in revised form 15 June 2012

Accepted 25 June 2012

Available online 29 June 2012

Keywords:

IgE

Human

Allergen-specific

Basophil

Dendritic cell

Food allergy

ABSTRACT

Since the discovery of IgE in 1967, there has been an evolution in design and quality of immunological methods used to research allergic disease mechanisms, to diagnose allergic disease in the clinic, and to monitor allergic patients on various therapeutic regimens. This issue of the Journal of Immunological Methods highlights recent methodological developments in three areas: (1) understanding of the interactions between T-cells, dendritic cells and B-cells and various signaling mediators in the induction phase of sensitization, (2) developments in the definitive diagnosis of allergic disease with a focus on food allergy and eosinophil related diseases, and (3) enhancements in allergy patient management through improved methods for monitoring allergen levels in the environment and documenting changes in IgE in patients on therapeutic anti-IgE (Omalizumab). This special issue of the Journal of Immunological Methods examines each of these three areas and provides a compendium on state-of-the-art immunological methods used to assess human allergic disease.

© 2012 Elsevier B.V. All rights reserved.

Contents

1. Introduction	1
2. Cellular modulation of allergic responses	2
3. Immunological methods in food allergy and eosinophil related diseases	2
4. Assessment of environmental allergen and patients managed with anti-IgE (Omalizumab)	2
References	3

1. Introduction

Hypersensitivity denotes an exaggerated or inappropriate form of an adaptive immune response. Historically, Gel and Coombs delineated 4 categories of hypersensitivity: Type 1

(immediate-IgE antibody mediated), Type II (antibody dependent cytotoxicity-usually IgG antibody mediated), Type III (immune complex mediated) and Type IV (delayed-antigen-sensitized T cell mediated). This special issue of the Journal of Immunological Methods features a series of reports on humoral and cellular immunological methods that provide researchers with novel tools to investigate the mechanisms of allergic disease. In addition, immunological methods are discussed which facilitate the diagnosis and management of patients who have experienced IgE-mediated (Type I) hypersensitivity reactions.

* Johns Hopkins Asthma and Allergy Center, Johns Hopkins Dermatology, Allergy and Clinical Immunology (DACI) Reference Laboratory, Room 1A20, 5501 Hopkins Bayview Circle, Baltimore, MD 21224, USA. Tel.: +1 410 550 2031; fax: +1 410 550 2030.

E-mail address: rhamilt2@jhmi.edu.

2. Cellular modulation of allergic responses

IgE was identified as a unique immunoglobulin isotype in 1967 (Hamilton, 2005). Once IgE had been discovered as the gatekeeper of effector cell (mast cell and basophil) degranulation, scientists across the world began using various immunological methods to improve the diagnosis and management of patients with allergic disease. The first two articles in this series examine the interface between the cellular (T-cell, B-cell and dendritic cell) and humoral (IgE and IgG antibody and cytokines) immune response in allergic patients.

Lund et al. (2012) examine the critical role that IgE antibody-facilitated allergen presentation and T-cell activation play in maintaining the level of sensitization and effector cell mediator release. The report discusses how the concentration and specificity of IgE antibody and allergen and their interactions with cell surface-anchored immunoglobulin E receptors (more specifically the high affinity IgE-receptor FcεRI on basophils and mast cells, and the low affinity IgE-receptor FcεRII on B-cells) influence both mast cell and basophil activation and facilitated antigen uptake. Finally, they illustrate these relationships within the context of the diagnostic algorithm for allergic disease and the monitoring the effectiveness of allergen-specific immunotherapy. Frischmeyer-Guerrero and Schroeder (2012) extend this discussion of humoral and cellular component interactions by focusing on dendritic cell responses and their role in the sensitization phase and in altering the manifestations of allergic disease through cytokine mediated immunomodulation.

3. Immunological methods in food allergy and eosinophil related diseases

The next set of papers in this issue focus on the diagnosis of allergic disease, with an emphasis on food allergy and eosinophilic diseases. As a general background, immunologically-naïve humans develop immune responses to foreign substances such as foods, drugs and environmental allergens following repetitive exposure. A subset of individuals is genetically-predisposed to producing IgE antibody, which circulates and binds principally onto high affinity IgE receptors on effector cell surfaces in tissue (mast cells) and in circulation (basophils). At the point where IgE antibody can be detected in the blood or skin, the individual is considered “sensitized”. However, early in this period of becoming sensitized, the individual may not have had a sufficiently mature IgE antibody response in terms of its quantity or quality (e.g., specificity or affinity) to manifest clinically-evident allergic symptoms following a subsequent allergen exposure. With repetitive allergen exposure by inhalation, ingestion or injection, the concentration and affinity of the IgE antibody response increase, its specificity becomes more directed to unique allergenic epitopes and the IgE specific activity (percentage of total IgE that is specific for the allergen) increases (Christensen et al., 2008; Hamilton et al., 2010). Each of these events is associated with maturation of the individual's humoral immune response to the point where critical levels of allergen-specific IgE antibody bind onto the surfaces of mast cells and basophils. At the point where allergen exposure produces sufficient IgE antibody aggregation, vasoactive mediators may be released which can lead to the expression of a

spectrum of clinically evident allergic responses (e.g. rhinitis, hives, urticaria and anaphylaxis).

A positive clinical history of allergic symptoms is routinely supported by examining the patient for allergen-specific IgE antibody in the skin or blood (Bernstein et al., 2008). Occasionally, mismatches between a positive clinical history and a negative IgE antibody response in the skin and/or blood causes the clinician to request a provocation procedure to clarify the diagnosis. Jarvinen and Sicherer (this issue) overview the most widely used diagnostic provocation procedure, namely the oral food challenge. It is considered the gold standard for the diagnosis of food allergy and is useful in verifying whether an allergy to food exists. Oral food challenges are also used to monitor the patients for resolution (e.g. a developed tolerance or loss of sensitivity) to the food. This paper also discusses the predictive value of serological levels of food-specific IgE antibody since higher concentrations of food-specific IgE reportedly correlate with an increased likelihood of experiencing a reaction following ingestion of a food. The authors review other conditions in which an oral food challenge may be diagnostically useful, namely in non-IgE-mediated food allergy such as food protein-induced enterocolitis and mixed IgE and non-IgE mediated food allergy including atopic dermatitis and eosinophilic gastroenteropathies.

Na et al. (2012) in this issue extend the discussion to the management of patients with eosinophilic disorders. They overview the diseases in which eosinophils are a key cellular offender. They then report a novel immunological assay for the detection of soluble Siglec-8 in blood which has potential as a serological marker of eosinophil involvement in an allergic disease. They provide data to suggest that Siglec-8 may serve as a biomarker for discriminating among patients with the different eosinophil associated diseases.

4. Assessment of environmental allergen and patients-managed with anti-IgE (Omalizumab)

Management of patients with allergic disease is the subject of the remaining three papers in this issue. Avoidance is potentially the least expensive and most effective treatment which involves separation of the allergic patient from the allergen source. However, to avoid allergen, it is helpful to know that the level of allergen in a given environment is present in levels that are potentially injurious to the patient. This provides a rationale for justifying expensive remediation procedures. Allergen quantification can be subdivided into quantification of indoor and outdoor aeroallergens. Indoor allergen burdens are currently assessed in private homes and schools by laboratories which perform monoclonal antibody based assays for quantitative measurement of indicator allergens released from dust mites (Der p 1 and 2; Der f 1 and 2), cats (Fel d 1) and dogs (Can f 1) and rodents (mouse Mus m 1; rat (Rat n 1), cockroaches (Bla g 1 and 2) and fungi (viable mold spores colony counts). High levels of these allergens in the households of sensitized individuals signal consideration of remediation (Hamilton and Eggleston, 1997).

In some cases, effective avoidance has been shown to lead to a reduction in the individual's level of sensitivity and risk for symptoms (Hamilton and Brown, 2000). The paper by Rittenour et al. (2012) in this issue examines immunologic, spectrophotometric and nucleic acid based assay methods

for the detection and quantification of outdoor airborne pollen. Laboratories participating in the air monitoring station network currently use microscopic identification of pollen morphological phenotypes to identify and quantify pollen. Rittenour et al. discuss additional methods, limitations, required expertise and examiner bias that can optimize and enhance the semi-standardized microscopic approach to assess pollen burden and estimate exposure. Unfortunately, allergen avoidance is often not possible, especially for the majority of individuals who are sensitized to multiple allergen specificities or where it is difficult to identify an allergen's presence.

For those not managed with allergen avoidance, a second management strategy has been to reduce allergic symptoms with pharmacotherapy (e.g. anti-histamines, steroids). Treating the symptoms does not address the underlying allergic state, however, it can provide immediate relief. A third management modality involves immunotherapy in which the offending allergen(s) is(are) injected to induce a state of desensitization or tolerance. Possibly the most effective immunotherapy treatment involves purified *Hymenoptera* venom injections into an individual who is known to be sensitive to allergenic proteins in venoms from yellow jackets, yellow hornets, white-faced hornets, Polistes wasps and/or honeybees (Golden et al., 2011).

Finally, in 2003, the US Food and Drug Administration approved a novel humanized monoclonal anti-IgE therapy (Xolair [Omalizumab]) which binds to IgE and blocks its binding onto the high affinity IgE receptor on effector cells. The goal of this therapy has been to reduce the amount of “free IgE” or IgE that is unbound with Omalizumab in vivo so as to maximally block the binding and thus reduce the amount of IgE antibody bound to the surface of effector cells. The last two papers in this issue examine immunological methods that can be used to assess the levels of circulating free IgE (unbound with Omalizumab) in serum and changes in basophil function following Omalizumab administration. Hamilton et al. (2012), examines Law of Mass Action considerations for the design of immunological methods which permit the quantification of free (non-Omalizumab bound) IgE in the serum of patients receiving anti-IgE therapy. The rationale for and design and performance of a surface plasmon resonance assay for the detection of “free” IgE (unbound with anti-IgE) is presented as an alternative to microtiter plate based ELISAs. The final paper in this series from Saini and MacGlashan (2012) examines basophil mediator release methods that are useful in monitoring changes of basophil allergen reactivity following the administration of Omalizumab.

In summary, this special issue of the Journal of Immunological Methods provides an overview of the evolution in immunological methods used to investigate, diagnose and

treat patients with allergic disease. Automation, improved immunological reagents (more specific capture antibody and detection antibody reagents) and the identification of mechanistic pathways involved in the induction and blocking of mediator release from effector mast cells and basophils will all work together to ultimately improve the management effectiveness for allergic patients.

References

- Bernstein, I.L., Li, J.T., Bernstein, D.I., Hamilton, R.G., Spector, S.L., Tan, R., Sicherer, S., Golden, D.B., Khan, D.A., Nicklas, R.A., Portnoy, J.M., Blessing-Moore, J., Cox, L., Lang, D.M., Oppenheimer, J., Randolph, C.C., Schuller, D.E., Tilles, S.A., Wallace, D.V., Levetin, E., Weber, R., American Academy of Allergy, Asthma and Immunology, American College of Allergy, Asthma and Immunology, 2008. Allergy diagnostic testing: an updated practice parameter. *Ann. Allergy Asthma Immunol.* 100, S1.
- Christensen, L.H., Holm, J., Lund, G., Riise, E., Lund, K., 2008. Several distinct properties of the IgE repertoire determine effector cell degranulation in response to allergen challenge. *J. Allergy Clin. Immunol.* 122, 298.
- Frischmeyer-Guerrero, P.A., Schroeder, J.T., 2012. Cellular Immune Response Parameters that Influence IgE Sensitization. *J. Immunol. Methods* 383, 21.
- Golden, D.B., Moffitt, J., Nicklas, R.A., Freeman, T., Graft, D.F., Reisman, R.E., Tracy, J.M., Bernstein, D., Blessing-Moore, J., Cox, L., Khan, D.A., Lang, D.M., Oppenheimer, J., Portnoy, J.M., Randolph, C., Schuller, D.E., Spector, S.L., Tilles, S.A., Wallace, D., Joint Task Force on Practice Parameters, American Academy of Allergy, Asthma & Immunology (AAAAI), American College of Allergy, Asthma & Immunology (ACAAI), Joint Council of Allergy, Asthma and Immunology, 2011. Stinging insect hypersensitivity: a practice parameter update 2011. *J. Allergy Clin. Immunol.* 127, 852.
- Hamilton, R.G., 2005. Science behind the discovery of IgE. *J. Allergy Clin. Immunol.* 115 (3), 648.
- Hamilton, R.G., Saini, S.S., MacGlashan, D., 2012. Surface plasmon resonance analysis of free IgE in allergic patients receiving Omalizumab (Xolair). *J. Immunol. Methods* 383, 54.
- Hamilton, R.G., Brown, R.H., 2000. Impact of personal avoidance practice on healthcare workers sensitized to natural rubber latex. *J. Allergy Clin. Immunol.* 105, 839.
- Hamilton, R.G., Eggleston, P.A., 1997. Environmental allergen analyses. *Methods* 13, 53.
- Hamilton, R.G., MacGlashan Jr., D.W., Saini, S.S., 2010. IgE antibody-specific activity in human allergic disease. *Immunol. Res.* 47, 273.
- Jarvinen, K.M., Sicherer, S.H., 2012. Diagnostic oral food challenges: procedures and biomarkers. *J. Immunol. Methods* 383, 30.
- Lund, G., Willumsen, N., Holm, J., Christensen, L.H., Würtzen, P.A., Lund, K., 2012. Antibody Repertoire Complexity and Effector Cell Biology Determined by Assays for IgE-mediated Basophil and T-cell activation. *J. Immunol. Methods* 383, 4.
- Na, H.J., Robert, G., Hamilton, R.G., Klion, A.D., 2012. Bochner BS Biomarkers of Eosinophil Involvement in Allergic and Eosinophilic Diseases: review of phenotypic and serum markers including a novel assay to quantify levels of soluble Siglec-8. *J. Immunol. Methods* 383, 39.
- Rittenour, W.R., Hamilton, R.G., Beezhold, D., Green, B.J., 2012. Immunologic, Spectrophotometric and Nucleic Acid Based Methods for the Detection and Quantification of Airborne Pollen. *J. Immunol. Methods* 383, 47.
- Saini, S.S., MacGlashan Jr., D.W., 2012. Assessing basophil functional measures during monoclonal anti-IgE therapy. *J. Immunol. Methods* 383, 60.