

Chapter 10

Immune Responses to Allergens in Atopic Disease: Considerations for Bioinformatics

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Abstract Allergies are mediated by several immunological mechanisms that make different contributions in different patients. Measurements of T_H2 and regulatory T cell responses are poorly standardized and are highly influenced by cell culture conditions and allergen preparations. There are, however, purified allergens and new techniques that can facilitate direct ex vivo measurements and purified allergens for use. IgE antibodies can be accurately and reproducibly measured using pure allergens that represent the important specificities and allergen arrays can be used to identify cross-reactivities, and hence, reduce false positives. IgE binding to most allergens is dominated by a few components that usually bind with the same pattern for most patients, although there are exceptions that are now recognized to provide important information. Measurements with allergen arrays can also reduce the underestimation of antibody titers due to underrepresentation of the important allergen components. Furthermore, IgE titers should be analysed preferably as continuous variables or if cut offs are used for thresholds the titers should reflect a high probability of substantive atopy and these titers differ for different allergens. The IgE value that has historically been used to indicate reaction sensitivity, 0.35 IU/mL, is the historic reaction sensitivity for IgE measurements and is a trivial amount of antibody for many allergens but a significant amount for others. Positive skin test responses can be readily elicited in subjects with trivially low IgE titers and no history of allergic disease and are thus a poor measure of atopy. The type of immune responses elicited by different allergens, as shown by IgE and IgG subclass titers and, from limited data, T cell responses to different allergens differ in memory cell phenotypes and cytokine profiles. There is much from these considerations that can be applied to improve the design and analysis of bioinformatic studies.

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10.1 Introduction

While the participation of T_H2 cells and IgE antibodies in atopy and atopic diseases needs no introduction, the relative roles of IgE and T_H2 -cell cytokines in the immunopathology of allergy are not well understood, and are the influences of regulatory and ancillary non- T_H2 responses. Type 1 allergic responses occur quickly with, for inhalant allergy, smooth muscle constriction, edema, and mucous secretion produced within 30 min of encountering the specific antigen. For many people this is followed by late reactions and chronic tissue damage. In skin tests, the late phase reactions show as swelling and erythema occurring after 2–6 h and are associated with mixed inflammatory cell infiltration. This can result not only from the secretion of T_H2 cell cytokines but also from the release of TNF- α (Grimbaldeston et al. 2006), IL-33 (Hsu et al. 2010) and lipid mediators (Pettipher 2008) from mast cells. Separate from the acute responses, T_H2 cytokines have direct chronic effects on inflammation, such as IL-5-mediated tissue eosinophilia, IL-13-mediated fibrosis and mucin induction. Insights into the mechanism of human atopic disease are now being obtained with new types of therapy and with gene expression and genome association studies.

Therapeutic trials with the anti-IgE monoclonal antibody omalizumab and monoclonal antibody antagonists of T_H2 cytokines are now showing the importance of the different responses in different patients. Direct T cell-mediated disease has been demonstrated by the ability of anti-IL-13 antibody (Scheerens et al. 2014) to inhibit late phase responses and improve lung function with little effect on IgE or immediate responses. Subjects that respond best to anti-cytokine treatment have high levels of periostin (Corren et al. 2011), a T_H2 -cytokine-induced tissue matrix protein. The beneficial effects of omalizumab for the treatment of immediate hypersensitivity have been repeatedly demonstrated although typically only 30 % of subjects obtain a worthwhile benefit (Holgate 2013). This monoclonal antibody can also reduce periostin levels (Parulekar et al. 2014), possibly by inhibiting the ability of IgE to enhance antigen presentation to T_H2 cells.

Studies of gene expression in T cell responses to allergen extracts have shown the activation of many T_H2 and other genes in inflammation-linked pathways (Bosco et al. 2006; Zhao et al. 2012), but there has been little consensus or follow-up and none have been examined responses to purified allergen components or directly compared allergens from different sources. Studies examining epithelial cells of asthmatics have been more informative, with the standout discovery being the identification of periostin expression (Woodruff et al. 2007), a valuable biomarker of asthmatic responses (Takayama et al. 2006).

Four of the five most consistent associations found in genome-wide association studies (GWAS), namely IL-33, thymic stromal lymphopoietin (TSLP), IL-1RL1, the IL-33 receptor (ST2) and HLA-DQ, strongly implicate T_H2 responses (Ober and Yao 2011) with the remaining 17q21 association implicating a gene for tissue remodeling. Other less universal associations with IL13, IL2RB, and SMAD3 similarly implicate T_H2 cells and another, RORA, group 2 innate lymphoid cells. Two others, SLC22A5 and PYH1N1, implicate epithelial cells and macrophages, respectively. Although these associations point to potentially important elements in atopy, they are limited because, from different estimates, they only account for 4–24 % of the known inheritance of asthma (Lee et al. 2011). Whole genome sequencing might reveal some missing polymorphisms but the missing inheritance is most likely due to gene-gene interactions and even interactions of only two genes can create problems for the current computational platforms. Another anomaly is that the genes identified as important for asthma by GWAS have little overlap with those important for atopy. Accordingly, a meta-analysis for GWAS for atopy revealed the importance of TLR6, STAT6, HLA-DQB1, IL-1RL1, LPP, IL-2, and HLA-B (Bonnelykke et al. 2013). These genes are important for immune function in general, and STAT6 for T_H2 responses, but only IL-1R1 and HLA-DQ were associated with asthma.

A feature of the analyses of therapy, gene expression, and genetic associations is that they have paid only limited attention to the size of the immune responses associated with atopic disease and none to the different types of immune responses induced by different allergens. Thus, there is a considerable scope for further studies with more informed designs and more informative measurements for quantitative analyses. Points that are critical for a better understanding of how atopy is measured and the varying nature of atopic responses to different allergens will be presented below.

10.2 Measurement of Atopy

10.2.1 *IgE Antibody Binding*

IgE binding has been most widely measured by the proprietary immunoabsorption test first released in 1974 as the Phadebas RAST and now the Phadia ImmunoCAP test. Absolute (gravimetric) measures of IgE binding could be derived from RAST tests but direct calibration was introduced in 1990 with the attachment of anti-IgE antibodies to the immunoabsorbent matrix to capture IgE standards (Yman 2001). The measurements are given in International Units (IU) of IgE based on a WHO reference preparation with 1 IU IgE now known to be 2.42 ng. All the early tests and current standard tests use unfractionated extracts made from sources of the allergens to bind IgE antibody. Since an excess of allergen on the solid phase matrix is essential for the quantitation of these assays, the underrepresentation

of allergen components and a lack of knowledge of the components present in extracts are important issues. Phadia tests with selected purified or recombinant allergens are now available but for the most part use components that are already well represented in extracts. Other proprietary assays like the Hycore allergen fluorescence system from HYTEC and Immulite 2000 3gAllergyT system also give absolute measurements.

Microarrays or allergen chips that give a profile of IgE binding to some of the allergen components from different allergens are now available. They have considerable clinical utility in distinguishing cross-reactivities that confound diagnosis and in some cases the profiles show if the suspected allergen is likely to cause mild or severe disease (Canonica et al. 2013). The Phadia ImmunoCAP ISAC measures IgE antibodies to a panel of 112 components from 51 allergen sources. Technically, thousands of components could be arrayed on a chip but the number has been limited by their known clinical utility and performance in multiplex assay conditions. Although stated as arbitrary, the ISAC Standardized Units (ISU) have been calibrated against standards characterized by ImmunoCAP assays and a good concordance of titers determined with microarrays and ImmunoCAP has been found for grass allergen components (Jahn-Schmid et al. 2003). A chip developed from the European Union-funded project MeDALL has multiplexed 170 allergen components coated in minute quantities (Lupinek et al. 2014). It has good titration characteristics for IgE concentrations up to 300 ng/mL but significant inhibition, not found for ImmunoCAP (Squire et al. 1986), occurs with physiological levels of IgG antibody. Other old (Jahn-Schmid et al. 2003) and new formats (Suzuki et al. 2011), however, do not show any indication of IgG interference so high-density quantitative arrays appear feasible.

Other quantitative IgE binding assays have been validated. One method that can be applied to microtiter-plate ELISA and has been used extensively for international reference sera for infectious disease (Holder et al. 1995), is to perform parallel assays of a test antigen and a heterologous standard. Providing the antibodies have similar affinity, as shown by titration curves, titers can be interpolated from the standard. Humanized monoclonal anti-Der p 2 antibodies have been developed for standards with standard binding of the allergens to the solid phase being accomplished by capture with the equal concentrations of purified monoclonal antibodies for each allergen (Ichikawa et al. 1999; Trombone et al. 2002) or, for recombinant components, with the use of monoclonal antibodies to epitope-tags (Hales et al. 2004, 2006). When conducted with Fel d 1 (Hales et al. 2013a; Ichikawa et al. 1999), Der p 1&2 (Trombone et al. 2002) and with a panel of house dust mite (HDM) components (Hales et al. 2006), the titers show good concordance with ImmunoCAP and for Fel d 1 also with titers determined by radioimmunoassay (Ichikawa et al. 1999). Unpublished work-up of the assays used by Hales et al. (2013a, 2006) showed no effect from pre-absorption of allergic sera with protein G to remove IgG antibodies.

10.2.2 IgE Antibody Predictive Values

The titer of 0.35 IU/mL of IgE antibody (2.42 ng/mL), which is frequently taken as a measure of atopy, is the historic lower limit of IgE that could be reliably detected by RAST test (Yman 2001) and has no biological significance. Indeed, IgE antibody titers of over 0.35 IU/mL to single protein antigens of *Haemophilus influenzae* and *Streptococcus pneumoniae* bacteria are found in 40–50 % of non-atopic subjects (Hollams et al. 2010) and, as shown for *H. influenzae*, these bacteria induce T_H1-biased responses with little IL-5 but with detectable IL-13 (Epton et al. 2002a). The limitation of the 0.35 IU/mL cut-off is particularly evident in population samples as shown in an unselected Finnish cohort (Haahtela and Jaakonmaki 1981) where allergic symptoms were found in only 40 % of subjects with detectable IgE antibody compared to 30 % of subjects without detectable IgE. Titers, over 17.5 IU/mL were, however, highly associated with allergic symptoms and titers below 3.5 IU/mL with asymptomatic subjects.

More recent European compilations found that, typically, 10 % of subjects with 0.35 IU/mL of IgE antibody to an aeroallergen would have been diagnosed with allergy by a physician with the probability increasing linearly with the log of the titer until about 3.5 IU/mL where it attained 80–100 % (Soderstrom et al. 2003). There are similarly shaped probability curves for the diagnosis of milk, egg, fish, and peanut allergy in children except that they plateau at over 17.5 IU/mL where 75–90 % probabilities are evident. A diagnosis of atopy in infants is however made with lower IgE titers (Urisu et al. 2014).

The probability that 5-year old children will have asthma symptoms shows a similar association with IgE titers. For HDM the probability of having allergic disease for subjects with an IgE titer of 0.35 IU/mL is 15 % and this slowly increases to a plateau level of only about 55 %, even for children with very high titers for example 300 IU/mL (Simpson et al. 2005). The probability curves for children with cat and dog allergies are however sharper reaching 85 % and 90 % disease prevalences at the higher antibody concentrations, thus showing greater penetration of disease. Analysis of children with seasonal rhinoconjunctivitis showed that the relationship with grass pollen allergen titers and disease resembled that for the cat and dog, with the curves reaching an 80 % disease prevalence (Marinho et al. 2007).

Some studies provide an important perspective on what might be expected from investigations that use unselected cross-sectional population samples. Women in a pregnancy cohort in Michigan USA, a state with similar prevalences of allergies to most temperate environments, showed a 46 % incidence of having an IgE titer of over 0.35 IU/mL to an allergen in the absence of symptoms compared to the incidence of 66 % in symptomatic subjects (Abraham et al. 2007). When analysed for different allergens, IgE was found in 10–20 % of the asymptomatic subjects, depending on the allergen, compared to 20–50 % for the symptomatic subjects. For cat, dog, and horse allergy, where some self-awareness of symptoms would be expected, 14.5 % of asymptomatic subjects had titers of over

0.35 IU/mL. In Europe, titers of asymptomatic subjects have indicated cut-offs of approximately 10 IU/mL for pollens and HDM (Pastorello et al. 1995), but 25–40 % of symptomatic subjects had titers below this value and 25–30 % of asymptomatic subjects had titers above. The relationship of IgE titer with asymptomatic sensitization also should consider that it often precedes symptomatic allergy (Bodtger et al. 2003).

10.2.3 Skin Prick Tests

Skin prick test (SPT) reactions are hypersensitivity reactions and, thus, a direct measure of allergenicity. They have been demonstrated with many purified and recombinant allergens (Niederberger et al. 2014), including minor allergens, so it is important to consider their relationship with allergic disease and atopic sensitization. For aeroallergens, SPTs can be readily demonstrated in subjects that have less than 0.35 IU/mL of IgE antibody (Haahtela and Jaakonmaki 1981). For example, in a teenage cohort, only 84 % of subjects with positive SPTs to grass pollen had over 0.35 IU/mL of IgE antibody and this number fell to 62 % for cat extracts. Conversely, only 3 % of subjects with detectable IgE were SPT negative. A contemporary investigation showed that, depending on the allergen, between 2.5 and 19.4 % of the IgE titers of SPT positive subjects were between 0.1 and 0.35 IU/mL. The association of SPT with allergic disease is also only weakly positive. In a cohort of unselected Finnish teenagers (Haahtela et al. 1980) that conducted SPTs with 12 inhalant allergens, only 61 % of the SPT positive subjects compared to 23 % of the negative subjects had allergic symptoms, and conversely 72 % of the symptomatic and 33 % of the asymptomatic subjects had positive SPT reactions. Contemporary multicenter studies with wider panels of aeroallergens (Haahtela et al. 2014) showed similar results, although subjects with larger SPT wheals had better associations with allergic disease. Similar analyses for food allergens have established larger wheal sizes to improve the specificity of the prediction of egg, milk and peanut allergies (Niggemann et al. 2005).

10.2.4 Allergen Provocation

Advances in protein isolation and recombinant DNA technology have allowed provocation studies that can measure the response of the target tissues using molecularly defined allergen components. This has provided dose response characteristics that can be used to define the importance of different allergen components.

Nasal provocation with Bet v 1 showed that symptoms were produced by low doses of allergen, usually under 1 µg in moderately allergic subjects (Godnic-Cvar et al. 1997; Niederberger et al. 2001; Tresch et al. 2003). The response to

the provocation correlated with the IgE titers but the subjects with low titers of 2–3 IU/mL still produced significant reactions (Tresch et al. 2003). Similar doses (10–100 µg) of Art v 1, the serodominant mugwort allergen, induced nasal symptoms with both natural and recombinant allergens and there were weak correlations with serum IgE and skin test reactivity (Schmid-Grendelmeier et al. 2003). A comparison of IgE titers and nasal provocation tests using different allergen components (Niederberger et al. 2001) found that a regimen with a top dose of 0.6 µg/mL resulted in consistent nasal responses to Bet v 1, Bet v 2, Phl p 1, Phl p 2, and Phl p 5, but that the responses to provocation with Phl p 2 and Bet v 2 far exceeded those expected from their IgE titers. Indeed, some subjects with highly positive skin and nasal provocation tests did not have detectable IgE antibody, even for allergens other than Phl p 2 and Bet v 2.

Conjunctival challenge with Bet v 1 (Arquint et al. 1999) showed that 0.15 µg of allergen could consistently induce symptoms. Similarly, natural date profilin, which cross-reacts extensively with profilins from many pollens and foods, produced large conjunctival reactions in grass-allergic subjects with as little as 5 µg/mL of allergen (Nunez et al. 2012).

Bronchial challenge with purified natural Der p 1&2 from HDM has been shown to cause immediate hypersensitivity symptoms, as measured by lung function tests, with doses of only 0.1 µg of allergen, although responses were more prevalent with doses increasing up to 1 µg. The responses were the same as those induced by HDM extracts containing the equivalent amount of allergen (Van Der Veen et al. 2001). The size of the immediate changes in the forced expiratory responses did not correlate with IgE titers and maximal responses were found in subjects with titers of only 3 IU/mL while typical IgE titers of HDM allergic subjects are 50–200 IU/mL (Van Der Veen et al. 1998). Differing from the immediate responses, larger late phase responses were found to HDM extract, indicating the importance of other allergen components, but this interpretation could be a function of the experimental design because in this study subjects that produced large immediate responses to low doses of pure allergen were not given higher doses that might have been required to elicit the late phase responses. Bronchial responses of grass pollen sensitized subjects have also been demonstrated with µg doses of profilin (Ruiz-Garcia et al. 2011), an allergen component that induces IgE titers of only a few IU/mL compared to the 50–100 IU/mL titers seen with the serodominant grass allergens. Its presence in amounts 1/10–1/100 less than these allergens, however, would be considered clinically significant.

Allergic responses to challenge with food allergen components have been accomplished using the apple PR-10 protein Mal d 1 (Bolhaar et al. 2005) in patients with birch pollen-related apple allergy. A double-blind placebo-controlled food challenge (DBPCFC) produced allergic symptoms with 10 µg, whereas a genetically engineered mutant with half the IgE binding capacity required tenfold more allergen for the same response. Oral provocation with profilin showed that even lower doses-induced oral and systemic, respiratory tract symptoms in food-allergic patients in a region with high grass exposure (Alvarado et al. 2014). In this

study, 0.074 μg profilin induced reactions in 22 % of patients and 7.2 μg profilin induced reactions in 50 % of patients, with some requiring rescue medication.

Provocation with single allergen components does not take into consideration the importance of a single component within a mixture where components can cooperatively cross-link IgE receptors. Cooperation has been demonstrated in an in vitro model using synthetic allergens and monoclonal antibodies (Handlogten et al. 2014) but, importantly, this was observed only when IgE titers were low. When high amounts of allergen were used, however, co-cross-linking was markedly inhibitory. Given, the simplicity of the experiments and the availability of recombinant allergens, it is surprising that no similar real-life experiments could be found for human allergy.

10.3 IgG Responses

IgG antibody has often been thought to have the capacity to protect from disease. Its production in allergy-tolerant beekeepers has been cited as an example, but a recent analysis has shown that their high IgG4 titers are accompanied by a paucity of IgE (Varga et al. 2013). A protective association in the presence of high titers of IgE antibodies was shown for HDM allergic children admitted to a hospital emergency department for asthma exacerbation (Hales et al. 2006). These children had very little IgG1 or IgG4 antibody in conspicuous contrast to children with stable asthma and similar IgE antibody titers. Further investigation (Hales et al. 2009) found that the lowest IgG1 or IgG4 anti-HDM titers were in children with recurrent and frequent asthma. A similar conclusion for cat allergy was drawn from a study of 5-year olds from two birth cohorts where a positive association between anti-cat IgE antibody titers and wheezing was found, but it was attenuated by IgG1 antibodies and not by IgG4 antibodies (Custovic et al. 2011). Persistent milk allergy is also associated with IgE without IgG1 and IgG4 antibodies (Ahrens et al. 2012). IgG antibody might be a marker for a less allergenic response or could act directly by blocking or sequestering allergen and by mediating negative Fc gamma receptor interactions.

10.3.1 IgE and IgG Antibody Patterns to Different Allergens

Different allergens induce distinctive patterns of IgE and IgG antibody (Table 10.1). HDM induce high IgE, IgG1, and IgG4 antibody titers in HDM atopic subjects but rarely induce IgG in non-atopic subjects (Hales et al. 2006). The IgG titers are higher for serodominant allergens with typical titers of 10 $\mu\text{g/mL}$ for IgG1 and 1 $\mu\text{g/mL}$ for IgG4, although it is complicated because IgG titers are low for subjects with recurrent asthma (Hales et al. 2006, 2009). Grass (Jutel et al. 2005; Rossi and Monasterolo 2004), tree (Harfast et al. 1998; Mobs et al. 2010) and

Table 10.1 IgE and IgG binding of common allergens^a

Allergen	IgE IU/ml	IgG1 μ g/ml	IgG4 μ g/ml	IgG in non-sensitized
House dust mite	80 (5–150)	10 (0–70)	1 (0–7)	No ^b
Grass Pollen	6 (3–300)	0.15 (0.01–0.3)	0.1 (0–0.6)	No
Birch Pollen	15 (3–60)	0.2 (0–0.6)	0.2 (0–0.3)	No
Ragweed Pollen	25 (6–83)	0.2 (0.1–0.3)	0.3 (0.25–0.45)	No
Cat ^c	10 (0–100)	2.0 (0–10)	0.2 (0–3)	Yes ^d
<i>Alternaria alternaria</i>	23 (1–50)	0.3 (0–0.6)	0.15 (0–0.3)	No
Bee venom	5 (2–11)	9 (5–17)	2 (1–11)	Yes in beekeepers
Peanut	40 (1–200)	20 (10–40)	8 (0.5–55)	Low/absent
Milk	16 (0–200)	4 (2–8)	3 (1–6)	Low/absent
Egg	6.0 (1–90)	8 (1–30)	0.5 (0.03–4.0)	Yes ^e

^aThe values are median titres and approximations of interquartile ranges (See text for references)

^bNo means that it is unusual to find IgG in subjects without IgE antibodies

^cCat median IgE titres vary from 18.2 (2–100) in asthmatic children to 2.9 (<0.35–20) for adults with rhinoconjunctivitis

^dYes but more prevalent in sensitized

^eYes but higher in sensitized children

weed (Creticos et al. 2014; Peebles et al. 1998) pollens induce IgE titers similar to HDM but induce very little IgG1 antibody and only low, under 1 μ g/mL, titers of IgG4, which is seen only in atopic subjects. IgE and IgG responses to fungi, as exemplified by those to *Alternaria*, are similar to the responses to pollens (Lizaso et al. 2008; Vailes et al. 2001). The IgE titers to cat allergens vary greatly from patient to patient and differences have been found for different clinical presentations. Subjects with rhinoconjunctivitis have repeatedly been shown to have low IgE titers, often lower than 1 IU/mL (Gronlund et al. 2008; Hales et al. 2013a; Linden et al. 2011; Smith et al. 2004; Worm et al. 2011) while asthmatic children show higher titers, similar to those seen with pollens (Gronlund et al. 2008). A clear difference with other common allergens is that many non-atopic subjects have high IgG1 and IgG4 antibody responses to cat allergens (Hales et al. 2013a; Platts-Mills et al. 2001). Cat allergen Fel d 1 induces IgG1 and IgG4 in allergic and non-allergic subjects while the other cat allergens only induce IgG4 and in much lesser amounts in the non-allergic subjects (Hales et al. 2013a). The IgG titers to cat in atopic patients, typically 2 μ g/mL for IgG1 and 0.2 μ g/mL for IgG4, are less than those to HDM but higher than that seen to pollens and fungal spores. IgE titers to bee venom allergens in atopic subjects, usually 2–10 IU/mL, are lower than those to HDM and pollen but the IgG1 and IgG4 titers are higher, similar to HDM (Varga et al. 2013) and are also found in nonallergic beekeepers, although not in people with limited exposure. For food allergies, peanut allergy is associated with high IgE titers to Ara h 1&2 (Eller and Bindslev-Jensen 2013; Hong et al. 2012), similar to HDM, and high anti-peanut IgG1 and IgG4 titers that are associated with IgE production (Hong et al. 2012; Tay et al. 2007). The IgE titers found in

milk-allergic (Shek et al. 2005) and egg-allergic (Ahrens et al. 2010) subjects are similar to those seen to pollen allergens but the anti-milk and anti-egg responses differ in that, although they both induce high IgG1 and IgG4 antibody titers, the IgG is associated with IgE titers for milk (Shek et al. 2005) but not to the same degree for egg (Ahrens et al. 2010; Tay et al. 2007).

10.4 T Cells

10.4.1 Associations of T Cell Responses with Allergic Symptoms

The in vitro proliferation of peripheral blood mononuclear cell (PBMC) T cells stimulated with grass and birch pollen extracts was shown to be similar for symptomatic sensitized subjects and asymptomatic sensitized subjects but higher than that of non-sensitized subjects (Assing et al. 2006). However, the T cells from the symptomatic subjects released higher levels of T_H2 cytokines. A similar distinction between symptomatic and asymptomatic subjects could be made for HDM allergy when PBMC were stimulated with HDM extracts (Till et al. 1997), where IL-5 was induced only in symptomatic subjects. It should however be noted that for both the pollen and HDM, the IgE titers were much lower in asymptomatic people. Similar associations were found when comparing anti-HDM responses in asthmatic and non-asthmatic subjects in a birth cohort (Heaton et al. 2005). T cell stimulation also shows a potential for diagnosing milk allergy since casein has been shown to not only stimulate T cells from PBMC of allergic subjects to secrete IL-4 and IL-13 but also to do so in a manner inversely proportional to the dose of milk that the donors could tolerate (Michaud et al. 2014).

10.4.2 Measuring Anti-Allergen T Cell Responses

Allergen stimulation assays measure recall responses of T cells that have been sensitized to allergen presented by dendritic cells in the lymph nodes. Here, the T cells become either central, effector, or resident memory cells (Turner et al. 2014). Central memory cells migrate through the blood, reentering lymph nodes through high endothelial venules, while effector memory cells circulate via the blood and tissues, entering lymph nodes via the afferent lymph, having being programmed via dendritic cells to home to the tissue exposed to the sensitizing antigen. Resident memory cells do not recirculate but persist in the tissues originally exposed to antigen. They have been studied in human skin and surgically removed lungs (Purwar et al. 2011) as well as from biopsies from lung and small bowel transplantation patients (Turner et al. 2014). Studies of PBMC from allergic and

nonallergic individuals show they have effector and central cell responses to the pollen allergen Bet v 1, but mainly only central memory responses to HDM Der p 2 (Wambre et al. 2011). There is no information on resident memory cells.

While allergen-specific T_H2 responses can be detected in PBMC from allergic subjects, it is difficult to compare experiments from different laboratories and measurements of immunoregulatory responses also differ. For example, IL-10 release induced by purified Der p 1 (Macaubas et al. 1999) and Der p 2 (Hales et al. 2002) has been shown to be higher for PBMC from HDM allergic subjects than nonallergic subjects, while other studies of subjects sensitized to a variety of allergens using different culture methods have indicated the reverse (Akdis et al. 2004). More recent investigations have shown that IL-10 does not regulate T cell responses in Der p 1-stimulated in vitro cultures (Maggi et al. 2007) and that Der p 1 stimulated PBMC from non-atopic and atopic asthmatic subjects produce the same IL-10 responses (Wang et al. 2009). Some of discrepancies could be due to patient selection, differences in cell culture and the allergen preparations, which have not always been described (Akdis et al. 2004). In vitro culture systems lack the tissue-specific dendritic cells and in addition, the physiological milieu of cytokines can become non-physiologically depleted or concentrated in the course of the assays. Early detection of responses with techniques such as PCR might circumvent some of these problems, but not all regulation occurs via transcriptional control and tissue-specific cytokines would still be missing. Cell cytokine-secretion assays that detect cytokine-producing cells before they proliferate and differentiate would only detect effector memory cells. Methods that enrich T_H2 cells with markers such as CCR4 and CRT_H2 (Shamji et al. 2015) can help early detection, but the physiological relevance of responses in the absence of other cell types needs to be considered. Recent strategies such as the isolation of cells expressing CD154, the CD40 ligand which is transiently activated directly after allergen stimulation, are helping to focus studies on the immediate ex vivo responses (Campbell et al. 2010), and advances in multiparameter flow cytometry (O'Donnell et al. 2013), mass cytometry (Atkuri et al. 2015) and digital PCR (Hindson et al. 2011) are among the new techniques that can further improve this. The use of allergen-MHC tetramers (Van Hemelen et al. 2015) provides a method for directly isolating allergen responsive T cells, although the representative nature of the MHC restriction elements and the epitopes used need to be taken into account.

A major impediment to evaluating many of the studies of T cell responses is the type and quality of the allergen used. Extracts of the allergen source continue to be used despite being known to contain substances that modulate cytokine secretion, including not only microbial products but also nonallergenic proteins produced by the allergen source (Rockwood et al. 2013). Comparison of the gene expression of epithelial cells exposed to grass extract and purified Phl p 1 showed that the extract up-regulated 262 genes compared to 71 for Phl p 1. Only some genes were regulated by both stimuli with some being in the opposite direction (Roschmann et al. 2012). Purified natural and recombinant allergens from HDM (Hales et al. 2002), cat (Hales et al. 2013a), grass pollen (Wurtzen et al. 1998) and birch pollen

(Gafvelin et al. 2005; Mobs et al. 2010) have been shown to elicit ex vivo T cell cytokine responses, although the responses are not as large as those historically achieved with extracts.

10.4.3 T Cell Responses to Different Allergens

Direct comparisons using Der p 2 and Bet v 1 components found that they had not only sensitized different memory compartments but also induced cells to release different patterns of cytokines with a strong T_H2 bias for Bet v 1 and a mixed pattern of T_H2 cytokines with IL-10 and IFN- γ release for Der p 2 (Wambre et al. 2011). These results concur with prior studies of the allergens (Gafvelin et al. 2005; Hales et al. 2002; Mobs et al. 2010) although it should be emphasized that, although low, IFN- γ can be readily detected after Bet v 1 stimulation. The difference between HDM and birch not only provides an interesting avenue of research but also shows that measurements used to determine the degree of sensitization with one allergen might be different for another. There has been little analysis of differences between allergen components from one source. It was found that the less important HDM allergen component Der p 7 induced more IFN- γ than the serodominant Der p 1, while it induced the same proliferative and IL-5 cytokine release as Der p 1, even for subjects without anti-Der p 7 antibodies (Hales et al. 2000). A similar difference has now been revealed for cat allergy with the lipocalin allergens Fel d 4 and Fel d 7 but not Fel d 1 stimulating PBMC to release IL-10, with all of the allergens inducing similar levels of T_H2 cytokines (Hales et al. 2013a). Individual cytokine signatures for different allergen components, as well as a disconnect between IgE binding and T_H2 cytokine responses, have been reported for PBMC responses to the peptide epitopes of allergens. For example, in cockroach allergy, the epitopes of Bla g 5 epitope induced both IL-5 and IFN- γ , while Bla g 2 epitopes induced only IFN- γ (Oseroff et al. 2012), although, to be cautious, the study was confounded by biased contributions from individual subjects and no consideration was made of the physicochemical properties of the peptides.

10.5 Serodominant Allergen Components

Allergen components allow IgE binding assays to be conducted with known and optimal concentrations of reagents to provide accurate reproducible measurements. Indeed, the sum of the binding to different components often exceeds the binding measured with extracts, providing a more accurate assessment (see below). The pattern of binding to the components additionally provides information on the source of the sensitization, cross-reactivities, and, in a few cases, special types of sensitization. For common sources of allergen, the IgE titers to

a few immunodominant components far exceed those to other IgE binding components. Since it is well established in mice that large allergic responses to one component can stimulate allergic responses to bystander antigens (Blumchen et al. 2006; Cadot et al. 2010; Cunningham et al. 2012; Eisenbarth et al. 2004; Kulis and Burks 2015), low responses to bystander antigens would be expected to be prevalent and be of questionable significance.

For grass pollen, the summated IgE titers to separate components showed higher binding than that measured with extracts, and this was mostly due to binding to the group 1, 2, 5, and 6 allergens (Mari 2003; Rossi et al. 2001), with groups 1 & 2 and 5 & 6 being, respectively, biochemically related. These results were more significant because recombinant proteins were used that excluded IgE binding to cross-reactive carbohydrates. The importance of this pattern has recently been confirmed with the demonstration that a cocktail of these components bound 80 % of the IgE in 70 % of grass-allergic subjects and that subjects with proportionally larger contributions from other allergens had low total titers (Darsow et al. 2014).

For birch pollen, nearly all of the IgE binding to extracts in most subjects can be accounted for by binding to Bet v 1 (Moverare et al. 2002), although IgE from subjects allergic to other pollens bind cross-reactive allergens in birch extracts, and there is cross-reactivity with Bet v 1-like allergens found in tree pollen and foods.

IgE binding to a panel of HDM components showed that IgE binding to Der p 1&2 accounted for over 50 % of the binding to extracts and over 50 % of the summated binding to all the components (Hales et al. 2006). Most of rest of the IgE binding was accounted for by binding to the mid-tier components Der p 4, 5, and 7, which, when present, had titers proportional to Der p 1&2. Other components only sporadically had titers similar to the mid-tier components, and, from known comparative studies, it can be calculated that many of the other denominated components make little contribution (Thomas et al. 2010). More recently, the IgE binding to the group 21 allergens has been shown to be similar to that of the biochemically related group five components, and the pleotropin Der p 23 has been shown to bind IgE with titers similar to Der p 1&2 and with, despite some exceptions, a strong correlation with the titers to Der p 1&2 (Vrtala et al. 2014). The allergenicity of HDM proteins that continue to be described from genomic (Chan et al. 2015) and proteomic (An et al. 2013) studies requires absolute determinations of IgE binding and comparison with other allergens, noting the discrepancies already found between IgE binding and SPT tests (Chan et al. 2015) and between studies from different laboratories (An et al. 2013; Chan et al. 2015). Component-resolved diagnosis for HDM allergy has already had application demonstrating that Australian aborigines, once the subject for genetic and epidemiological studies, showed IgE binding to the HDM component amylase and not to the serodominant allergens (Hales et al. 2007). Exposure to scabies, which is often endemic in many countries, might be the culprit. IgE binding to Der p 10 and Der p 11 shows some usefulness in that, although Der p 10 (tropomyosin) binding is sporadic, it defines a subpopulation that has a very broad specificity of binding to

HDM components (Resch et al. 2011), and Der p 11(paramyosin) is a very minor allergen for subjects with inhalant allergy but is a major allergen for atopic dermatitis patients (Banerjee et al. 2015).

The mite *Blomia tropicalis*, important in highly populated tropical and subtropical regions, has biochemically different serodominant components from the *Dermatophagoides* spp. The evolutionary related Blo t 5 & 21 are the primary sensitizers and provide a method to distinguish *B. tropicalis* sensitization from sensitization to cross-reactive *Dermatophagoides pteronyssinus* allergens (Thomas et al. 2010).

The summated IgE binding to a panel of Bla g 1, 2, 4, 5, and 7 cockroach components (Satinover et al. 2005) often gave higher IgE binding titers than those measured with extracts, although 36 % of subjects were positive to extracts and negative to the components. This could be due to minor allergens or cross-reactivities since these sera typically had low IgE titers. The Bla g 2 and 5 were the most important allergens, followed by Bla g 5, but they do not show the universal dominance found for Der p 1&2. More recently described cockroach arginine kinase and protease allergens (Pomes and Arruda 2014) could be important from their prevalence but the IgE binding titers have not been reported.

As shown with a panel of allergen components, Fel d 1 contributes over 50 % of the IgE binding for 60 % of cat-allergic subjects (Hales et al. 2013a). The remaining cat-allergic subjects usually have one of either Fel d 2, 4, 7, or 8 as their highest IgE binding component often accounting for the majority of the IgE binding. It is therefore possible that there are subgroups of cat allergies defined by responses to different serodominant allergens, and this appears important in severe cross-reactive allergy to mammalian allergens (Konradsen et al. 2014). Atopic dermatitis patients with cat allergy also show disproportionately high IgE binding to Fel d 2 & 4 (Wisniewski et al. 2013), which, like Der p 11 for HDM allergy, indicates that there are different types of sensitization for different diseases.

Considering food allergen components, IgE antibodies are mainly directed to casein in the case of milk allergy (Shek et al. 2005), and to conalbumin and ovalbumin for egg allergy (Caubet et al. 2012; Everberg et al. 2011). For peanut allergy, Ara h 1&2, and to a lesser extent Ara h 3, contribute most of the binding (Eller and Bindslev-Jensen 2013; Hong et al. 2012).

A recent comprehensive study of the allergen components in bee venom allergy showed that although the phospholipaseA2 Api m 1 was the highest IgE binding specificity, demonstrating binding IgE binding in 72.2 % of subjects and usually with high titer, the components Api m 2, 3, 4, 5, and 10 also made large contributions (Kohler et al. 2014) and that Api m 3 or Api m 10 were the only IgE binding components for in 5 % of the patients.

10.6 Influence of the Characteristics of Allergen Components on Immune Response and Innate Interactions

Immune responses are initiated following either tissue damage or interactions with innate immune receptors, called pattern recognition receptors that have evolved to recognize molecules produced by pathogens. The two families of receptors that are important for non-infectious antigens are the Toll-like receptors (TLR) and the C-type lectin receptors (CTLR). It has been proposed, without definitive evidence that these and other actions of innate immunity are important for allergenicity, and, since these differ for different allergens (Table 10.2), there will be different pathways to sensitization.

Members of the TLR family found on antigen-presenting cells have distinctive specificities for lipids, nucleic acids and some proteins, and polysaccharides. For allergens, the recognition of lipoproteins and proteolipids by TLR-2 and TLR-4 are important. Historically, TLR were found to direct T_H1 type responses for protective immunity, but they also direct T_H2 responses in the lung (Tan et al. 2010). The high occurrence of lipid binding amongst important allergens might be due to the TLR pathway. There are, however, vast differences in the type of lipid binding by different allergens (Thomas 2014). Fel d 1 has an extremely small lipid binding cavity that only fits small hormone-like lipids, and the same is true for the salivary lipocalins like Fel d 4 and Can f 6. Others, such as the von Ebner gland lipocalins Fel d 7 and Can f 1 and the ML-domain protein Der p 2, have structures that bind much larger lipids.

The CTLR bind glycosylated allergens or allergens bound to glycans and glycolipids. Some just capture antigen on antigen-presenting cells while others modify TLR-initiated signals to direct the nature of immune responses. DC-SIGN engagement, for example, can inhibit T_H1 responses by blocking IL-12 production and Dectin-2 can stimulate the release of the T_H2 -stimulating cysteinyl leukotriene. The effects of CTLR signaling are, however, very dependent on the precise circumstances (Parsons et al. 2014; Wang et al. 2009), so only possible influences on allergenicity can be proposed. A very probable CTLR function would be to direct allergens to alternately activated antigen-presenting cells that have their inflammatory functions modified by T_H2 cytokines and have up-regulated CTLR. Table 10.2 shows the many glycosylated allergens and the carbohydrate ligands Phl p 1&2. Included in the list are Der p 2 and Phl p 5, previously thought to be unglycosylated but now known to have unusual forms of glycosylation (Halim et al. 2015).

Chitin can interact with both CTLR and TLR ligands and enhance T_H1 , T_H2 , and T_H17 responses (Da Silva et al. 2010). Chitin adjuvant activity could accordingly be responsible for the high allergenicity of the chitin-binding peritropin allergen Der p 23, and, although the chitinase-like allergens Der p 15 and Der p 18 are not potent sensitizers, they induce IgE titers that, unlike most other mite components, do not correlate with the titers to Der p 1&2 (Hales

Table 10.2 Interactions of allergens and innate immunity

Biochemical activity	Allergens	Mechanism	Evidence
Cysteine protease	Der p 1, Der f 1	Proposed cleavage of immunological receptors including CD23 and proteolysis mediated increase of mucosal permeability	Cysteine proteases have protease-dependent adjuvant activity for T _H 2 responses but despite being ubiquitous they are not typically allergenic or even immunodominant allergens for other mite families. Activity within the reducing milieu of the endosome is feasible as found for parasites
Serine protease	Pen c 13, Pen c 18, Asp f 13, Asp f 18	Increase inflammation via protease activated receptors (PARs)	Reported as prevalent fungal allergens but the IgE binding titres are not documented. The abundant serine protease of HDM are weak allergens
Kallikrein	Can f 5	Kallikreins have proinflammatory properties from the release of bradykinin from kininogen	Can f 5 induces very high IgE titres in some people and is similar to the major human semen allergen
Glycosylation (biochemically demonstrated)	Der p 1, Der p 2, Fel d 1, Art v 1, Bla g 2, Phl p 1, Phl p 5, Ara h 1, caseins, Der p 15	Focus allergens onto C-type lectin pattern recognition receptors to modulate Toll-like receptor signaling and to induce cysteinyl leukotrienes that activate type 2 innate immune cells	Plausible scenarios for inducing T _H 2-biased signaling especially through DC-SIGN, Dectin-1 and Dectin-2. Dectin-2 dependent induction of cysteinyl leukotriene in mice has been demonstrated with HDM extracts. High expression of CTLRs is found on alternatively activated macrophages in T _H 2 milieu
Proteolipid Lipidation	Not known	Bind innate receptors especially TLR-2 and TLR4 to activate dendritic cells	See lipoproteins
Carbohydrate ligand	Phl p 1, Phl p 2	As for glycosylation	As for glycosylation
Lipoproteins Lipid Ligand (proposed)	Der p 2, Der f 2, Bet v 1, Der p 5, Der p 7, Fel d 1, Fel d 7, Fel d 8, Can f 1	As for proteolipids	Many important allergens are lipoproteins. The ability of Der p 2 to sensitise mice by mimicking the lipid binding MD2 protein of the TLR-4 complex has been demonstrated
Chitin binding	Der p 23, Der p 15, Der p 18, Blo t 12	Allergens attach to chitin thus having adjuvant properties	Der p 23 is a recently recognized potent HDM allergen although the other allergens Der p 15 and 18 that would be expected to bind chitin are weak sensitizers. Blo t 12, molecularly similar to Der p 15, can bind chitin to increase its allergenicity in mice

et al. 2013b). Zakzuk et al. (2014) have shown that chitin complexed to the Blo t 12 chitin-binding protein of *B. tropicalis* can increase its ability to sensitize mice, providing experimental evidence for its binding and adjuvanticity.

The cysteine protease activity of Der p 1 has engendered speculation about its importance in allergic sensitization, but its role remains unproven (Thomas 2013). Cysteine proteases are highly susceptible to oxidative inactivation, and no activity has been found in HDM preparations, so is not clear how the activity would manifest in the oxidative extracellular environment. Moreover, despite being ubiquitous enzymes, cysteine proteases are rarely immunodominant allergen components. Enzymatically activated cysteine protease does, however, have a propensity to induce T_H2 responses in mice and, as found for parasite cysteine proteases; they could act within the reducing milieu of the endosome. The serine protease allergens of HDM are poor allergens (Hales et al. 2006; Thomas et al. 2010) and serine proteases from other sources are rarely immunodominant. From the prevalence of IgE binding, it appears that some fungal serine proteases are immunodominant, as indicated for the *Penicillium* spp. group 13 and 18 allergens (Shen et al. 2007) and the *Cladosporium herbarum* Cla h 9 (Poll et al. 2009), but this has yet to be demonstrated with IgE binding titers. The kallikrein Can f 5 allergen of dogs is a serine protease that does induce high IgE titers in some people (Mattsson et al. 2010) and could act via its hydrolytic activity on the kallikrein substrate kininogen that releases bradykinin that triggers the release of nitric oxide and leukotrienes.

Many immunodominant allergens are lipoproteins, suggesting that the possession of TLR2 and TLR 4 signaling properties enhances allergenicity, as well other lipid mediated functions (Bublin et al. 2014). Der p 2, a homologue of the MD-2 protein, could mimic the MD-2 function of binding lipopolysaccharide and delivering it to TLR-4 to activate antigen-presenting cells. It has been shown to induce a T_H2-type TLR-4-dependent response in MD-2 knockout mice (Trompette et al. 2009) and Der f 2 has been shown to bind lipopolysaccharide at high affinity using the amino acids equivalent to those used by MD-2 (Ichikawa et al. 2009). There is, however, still no evidence for its importance in humans where MD-2 is found in abundance on myeloid cells, although interestingly unless induced by IFN- γ it is absent on epithelial cells (Roy et al. 2011).

While the importance of the potential innate interactions above have not been demonstrated, the poor allergenicity of HDM ferritin (Chan et al. 2015; Epton et al. 2002b) and arginine kinase (Hales et al. 2006, 2007), which are produced in large amounts (Batard et al. 2006), suggests that more than abundance or the presence of a component in an allergenic source is required to induce high allergic responses.

10.7 Conclusions

Bioinformatics provides powerful tools to dissect out the immune mechanisms that lead to atopy and allergy. An important advantage is that human atopy is highly prevalent so it should be possible to effectively stratify and refine gene

expression and genetic associations, as proposed here particularly by the sensitizing allergen. HDM and grass pollen allergy are highly prevalent in many countries, thus, separate analyses can be made to remove the complications resulting from the inclusion of responses that are qualitatively and quantitatively different. In further analyses, the responses to different allergens can be compared rather than pooled so that the known differences might be exploited. Since the atopic responses to the common allergens are mainly directed to a few allergen components, and these can be used for reproducible measurements, quantitative trait GWAS can be applied to both IgE and IgG antibodies. The latter is associated with both sensitization and protection so the results could provide important information on the development of disease. A critical area for development is the establishments of reproducible T cell assays, especially those that can determine the responses of T cells without introducing in vitro artifacts, and, as for basic immunological investigations in mice, use defined pure allergens in known and reproducible quantities. The limited experiments to date comparing allergen components suggest that there is a wealth of information that can be gleaned by comparing the different types of responses to the different allergens. Much is being obscured in responses to extracts that are mixtures of unknown composition, often, like HDM extracts, having no relationship to natural exposure. There are recent technological advances in mass cytometry and digital PCR with that have the potential to provide better ex vivo measures of T cells that will allow assays with allergen components. It should also be noted that anti-cytokine and anti-IgE therapy indicate that not all atopic subjects follow the same path to disease, so subgroups should be expected. Clearly, biomarkers discovered from bioinformatic analyses can be used to analyse therapeutic trials, as periostin has been already. The defining test for allergy still remains challenge with antigen by the natural route of exposure, and it has been shown that, even for minor food allergens, this can be accomplished with small doses of highly purified natural and recombinant proteins. A direct relationship between the type of responses induced by allergen components after provocation and their immunological and bioinformatic profiles can thus be established. The use of molecularly defined provocation tests has severely declined in recent years (Niederberger et al. 2014), possibly due to regulatory processes, so the development of guidelines for its application that are acceptable to governing bodies and allergy societies should be recommended.

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Author Biography



Professor Wayne Thomas has been a Senior Research Fellow of the Australian National Health and Medical Research Council since 1987, a Senior Principal Research Fellow at the (recently renamed) Telethon Kids Institute, and Professor at the University of Western Australia since 1998. He investigated fundamental T cell biology at the Clinical Research Centre in London (1974–1983) and at the Walter and Eliza Hall Institute in Melbourne Australia during which time he was the first to demonstrate antigen-induced production of interferon gamma from CD4 T cells. His best-known accomplishments, however, have been in pioneering the development of recombinant allergens and to characterize house dust mite allergens. These have been used to explore formulations for immunotherapy that have led to the use of peptides representing allergens for immunotherapy, which have now been successfully used in clinical trials. More recent studies have extended knowledge of the spectrum of important cat allergens and how the responses that they induce are affected by single allergen immunotherapy with Fel d 1. He has also studied modified

notherapy, which have now been successfully used in clinical trials. More recent studies have extended knowledge of the spectrum of important cat allergens and how the responses that they induce are affected by single allergen immunotherapy with Fel d 1. He has also studied modified

allergens and the interaction of allergens with the innate immune system. Since 2007, in conjunction with Prof Belinda Hales, he has used defined recombinant bacterial and viral antigens to describe associations of antimicrobial immune responses in early life with the development of allergy and asthma leading to the exploration of possible mechanisms of the association between infection and allergy.