

Clinical Immunology Review Series: An approach to the use of the immunology laboratory in the diagnosis of clinical allergy

ARTICLES PUBLISHED IN THIS CLINICAL IMMUNOLOGY REVIEW SERIES

allergy in childhood, allergy diagnosis by use of the clinical immunology laboratory, anaphylaxis, angioedema, management of pulmonary disease in primary antibody deficiency, recurrent infections in childhood, recurrent infections in adulthood, recurrent oro-genital ulceration, recurrent superficial abscesses, urticaria, vasculitis/CTD

P. Williams,* W. A. C. Sewell,[†]
C. Bunn,[‡] R. Pumphrey,[§] G. Read*
and S. Jolles*

*Department of Biochemistry and Immunology, University Hospital of Wales, Cardiff, [†]Path Links Immunology, Scunthorpe General Hospital, North Lincolnshire, [‡]Department of Immunology, Royal Free Hospital, London, and [§]Department of Immunology, St Mary's Hospital, Manchester, UK

Accepted for publication 25 April 2008

Correspondence: P. Williams, Department of Biochemistry and Immunology, University Hospital of Wales, Heath Park, Cardiff CF14 4XW, UK.

E-mail: paule.williams@cardiffandvale.wales.nhs.uk

Summary

In the last 10 years UK immunology laboratories have seen a dramatic increase in the number and range of allergy tests performed. The reasons for this have been an increase in the incidence of immunoglobulin E (IgE)-mediated allergic disease set against a background of greater public awareness and more referrals for assessment. Laboratory testing forms an integral part of a comprehensive allergy service and physicians treating patients with allergic disease need to have an up-to-date knowledge of the range of tests available, their performance parameters and interpretation as well as the accreditation status of the laboratory to which tests are being sent. The aim of this review is to describe the role of the immunology laboratory in the assessment of patients with IgE-mediated allergic disease and provide an up-to-date summary of the tests currently available, their sensitivity, specificity, interpretation and areas of future development.

Keywords: allergy, diagnosis, IgE antibody

Introduction

The most important part of the initial assessment of a patient suspected of having allergic disease is a thorough clinical history and relevant examination in order to guide subsequent investigations. While the cause of allergic symptoms may be obvious because they follow within minutes of exposure to an allergen, often more than one possible allergen is suggested by the circumstances. The determination of which is the more likely cause of symptoms depends on allergy tests, which may include *in vivo* tests such as skin prick testing (SPT), laboratory-based *in vitro* analyses or challenge tests with the putative causative allergens.

Skin prick testing is a straightforward clinical test to support the diagnosis of allergy and is cheap, has an unrivalled sensitivity for the majority of allergens, and enables immediate availability of results to patients in a visible demonstration of reactivity. Results are expressed in mm of wheal diameter, and compared with that because of histamine at a standard concentration of 10 mg/ml (usually 3–5 mm).

Laboratory-based assays for specific immunoglobulin E (IgE) are feasible when patients are taking anti-histamines, when SPT may be difficult in the presence of dermatographism or eczema, and a precise, numerical result enables comparison with subsequent test results. Such laboratory tests do not require that reagents and clinical interpretation be performed in the clinic or at the bedside, as for SPT, and the serum used can be archived.

Although determination of specific IgE is an essential part of the investigation of anaphylaxis, the timing of samples may be relevant. It is not recognized widely that samples taken from patients during an episode of anaphylaxis may have reduced allergen-specific IgE levels significantly [1]. Although a positive result may be helpful at the time of an acute episode, an unexpected negative result should trigger a repeat blood test some weeks later.

While this review focuses upon *in vitro* testing for specific IgE (as this is by far the most frequently requested category of test), it is important to emphasize that there are numerous other areas in which the immunology laboratory can help in the investigation of patients presenting to an allergy service.

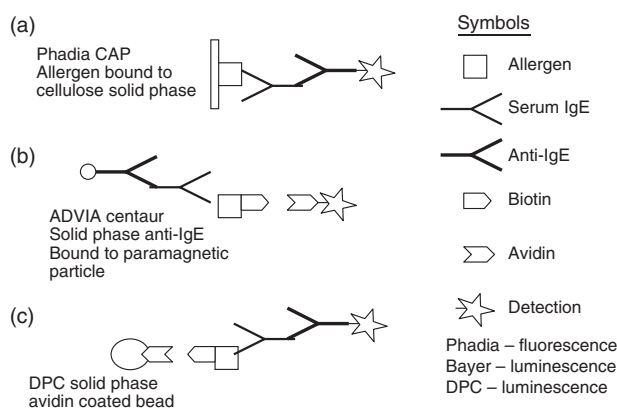


Fig. 1. Comparison of the three main methods currently used for the detection of specific immunoglobulin E (IgE).

Total and specific IgE testing

Assays for total serum IgE and allergen-specific IgE levels were developed initially in the late 1960s and early 1970s shortly after the discovery of the IgE class of Ig [2]. They used anti-IgE conjugated with radio-labelled iodine¹²⁵ to detect both total serum IgE and allergen-specific IgE bound to solid-phase immobilized anti-IgE or allergen, respectively, and the acronym radio-allergo-sorbent test [3,4] remains in use today, despite the current use of fluorescence or luminescence reporter systems.

Within the United Kingdom, the immunoassay market is dominated by the Phadia Immunocap system (Phadia AB, Uppsala, Sweden) [5], but with a significant presence from the Diagnostic Products Corporation Immulite (Euro/DPC Limited, Glyn Rhonwy, Llanbevic, Gwynedd, UK) [6], Bayer ADVIA (Bayer PLC, Bayer House, Strawberry Hill, Newbury, Berks, UK) [7] and, to a lesser extent, the Hitachi CLA [Techno-Path (Distribution) Ltd, Rosse Centre Holland Road, National Technological Park, Plassey, Limerick, UK], formerly the multi-allergen specific IgE test (MAST) [8] systems. All now operate on fully automated analysers utilizing stored calibration curves that are therefore capable of producing quantitative results. The Immunocap method uses a high surface area solid phase polymer (CAP) to bind a high density of allergen. The Immulite system is a four-step chemiluminescent assay using biotinylated allergens in a liquid phase coupled to ligand-coated beads. The ADVIA system also employs liquid phase biotinylated allergen in an IgE capture sandwich immunoassay (Fig. 1).

Testing for allergen-specific IgE is carried out currently in approximately 100 UK laboratories, whose workload varies from 5000 to 50 000 tests/year [9]. Each laboratory can choose from a repertoire of about 800 allergen preparations available as panels or individual allergens (depending upon the manufacturer) falling into the following major sub-groups: (a) spores and pollens, (b) foods, (c) insects and mites, (d) venoms, (e) animal proteins, (f) drugs and (g)

occupational allergens. The complexity of allergen extracts and their standardization continue to be a major challenge in the further optimization of specific IgE testing. The production and characterization of recombinant allergens is a slow and laborious process, although progress is being made. A case in point is the latex allergen *Hevea brasiliensis* 5 (Hev b 5): as this labile protein may be lost in the manufacturing process, purified recombinant Hev b 5 is added to improve the sensitivity of *in vitro* testing [10].

The optimal use of resources for the diagnosis of allergy depends upon appropriate partnership between requesting clinicians and the immunology laboratory. It is difficult to know which tests to perform when 'allergy screen' or 'allergy testing please' are the only pieces of information on the request form accompanying a blood sample. Conversely, an extensive list of allergens not based on an accurate clinical history with a temporal relationship to symptoms is not ideal. In the interests of economy and reducing the number of false positive results, it may not be helpful to test for mixed panels of allergens when single allergen tests are indicated, as positives on mixed panel testing will trigger the need to retest each individual component. However, this depends upon the rate of negative findings: in a survey of requests emanating from general practitioners and ear nose and throat clinics in North Wales it was found more economical to screen with mixed CAPs and retest individual allergens in the few positives (personal communication, Richard Pumphrey).

Until recently, the numerical results obtained in assays for specific IgE antibodies were converted into, and reported by, laboratories as grades, in an effort to simplify their meaning for the clinicians requesting the tests. Most laboratories have changed (or are changing) their reporting scheme from the previous grades 1–6 to report the exact value in IU/ml. The reasons for the change are to avoid loss of quantitative information in IU/ml, which allows better prediction of the likelihood of clinical reaction following exposure to that allergen. During the current transition period, most laboratories will report results both in grades and in IU/ml. The relationship between the grades used, the IU/ml and a very rough estimate of the level of risk of developing symptoms after exposure to that allergen are shown in Table 1.

Table 1. Risk of allergic symptoms after allergen exposure as indicated by allergen-specific immunoglobulin E antibody grade.

Grade	IU/ml	Approximate risk of symptoms after exposure
0	< 0.35	Extremely unlikely
1	0.35–0.7	Mild, possible
2	0.7–3.5	Mild, more possible
3	3.5–17.5	Moderate, likely
4	17.5–50	Moderate, more likely
5	50–100	Very likely
6	> 100	Extremely likely

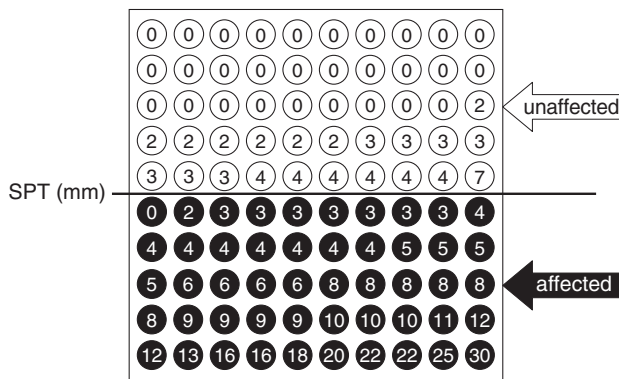


Fig. 2. Peanut skin prick test wheal diameter in equal-sized populations of affected and unaffected individuals. A majority of unaffected have negative results and a majority of affected have wheals greater than 5 mm. Wheal diameters of 3–4 mm are approximately equal in the affected and unaffected populations and therefore give no information about whether or not the patient is allergic.

Several manufacturers are now using extended control curves, which allow values for specific IgE to be reported below 0.35 IU/ml down to zero, so that this additional information is also available to clinicians.

The risk of developing symptoms following exposure to an allergen and the severity of any ensuing symptoms relate to the level of exposure to that allergen, the route of exposure and other factors. Thus a particular value in IU/ml of sIgE against one allergen (e.g. egg) does not necessarily equate in any terms to the same level of sIgE against a different allergen (e.g. peanut).

Sensitivity and specificity of tests for IgE-mediated allergy

When specific IgE tests are positive, does this mean that an allergic cause for the symptoms has been found, and when they are negative that the allergy is disproved? Unfortunately, the correlation between test result and allergic disease is imperfect. The fraction of those with the allergy that is detected by a test is its sensitivity, and the specificity is the fraction of those without the allergy who have negative results. Sensitivity and specificity are defined for a given cut-off value, below which the test is considered negative and above which it is considered positive. If the cut-off is raised, the test becomes more specific but less sensitive, and if it is lowered, the opposite happens. Plotting the sensitivity against specificity produces a receiver operator characteristic (ROC) curve; the shape of this curve reflects how good the test is at discriminating between affected and unaffected individuals.

Using a cut-off value for 'positive' simplifies the analysis but throws away useful information contained in the raw test results (mm diameter of SPT wheals or specific IgE in IU/ml). An alternative approach uses likelihood ratios [11]: this is explained most easily by considering a population

with equal numbers of affected and unaffected individuals (Fig. 2). The affected population has higher numbers of strongly positive results and the unaffected has higher numbers of negative results. Between these, there is a band of values present in approximately equal numbers in affected and unaffected; this is the region of the ROC curve with slope $\gg 1$; it is also the optimal cut-off for positive results. Because results in this band (commonly SPT around 2–4 mm wheal diameter or specific IgE in the range 0.5–2 IU/ml) occur equally probably in affected and unaffected patients, they give no information about whether or not the individual is affected by allergy; values below this band increase the probability that the patient is unaffected (often by a factor of 3–30 for an undetectable response), and higher values increase the probability the patient is affected; very strongly positive results may do this by a factor of over 1000.

The probability of being affected before the test is the prior probability; the probability afterwards is the posterior probability; the relationship between these may be portrayed in a nomogram [12] (Fig. 3). The prior probability of a random individual being affected is the prevalence: for example, the prevalence of peanut allergy may be approximately 0.005 (0.5%). As just discussed, a weakly positive SPT result of 3 mm in an asymptomatic individual does not alter the probability of peanut allergy. A history in an individual

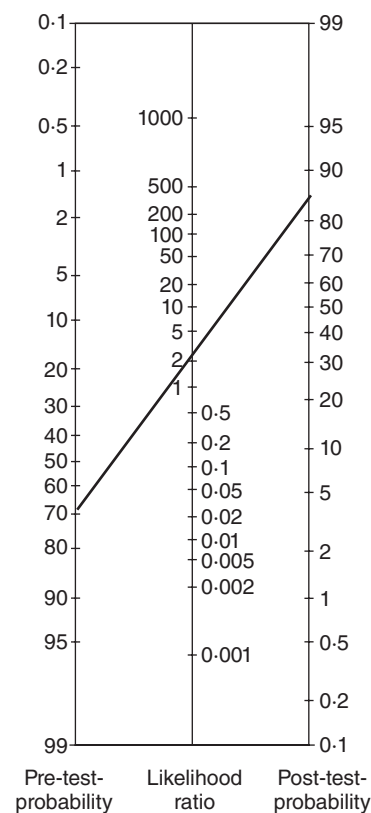


Fig. 3. Fagan nomogram for evaluating posterior probability from prior probability and likelihood ratio [12].

of repeated reactions immediately after eating or handling peanuts makes peanut allergy probable. If the prior probability of peanut allergy is assessed as 0.9, a 3 mm wheal still does not affect the probability of allergy and the posterior probability is still 0.9. The posterior probability following a negative test result might be 0.1 and following a strongly positive result 0.99.

Another way of looking at the same issue is to plot the probability of clinical allergy against the specific IgE to that allergen. Such a plot can be used to determine the specific IgE level above which the probability of clinical allergy is, for example, above 95% [13].

Allergy tests are at their most powerful when the prior probability is 0.5. For example, if an 18-month-old child has a typical acute allergic reaction after eating almond icing, the probability of nut allergy and egg allergy may each be 0.5. A strongly positive SPT to one and a negative test to the other allergen might lead to posterior probabilities of >0.95 and <0.05 respectively.

Likelihood ratios are independent of the prevalence of the condition tested, but not independent of clinical bias. If the subjects have atopic eczema and high total IgE, allergen-specific IgE is more likely to be detectable in those unaffected by this allergy: in other words, there will be more false positives. False negatives will be more common when affected individuals have a low total IgE. Unfortunately, the simple solution of using the ratio of specific : total IgE proved unhelpful in one study [14]; nevertheless, these factors need to be taken into account when evaluating the posterior probability.

Is there a gold standard in allergy diagnosis?

Patients have symptoms attributable to allergy in uncontrolled conditions that are often open to different interpretations. The clinical history may thus sometimes fail to influence the pretest probability in order to enable the measured value of the sIgE to the suspected allergen to allow a clinical diagnosis to be made with confidence. The sensitivity and specificity of a given level of allergen-specific IgE in diagnosing allergy varies between patients. As allergen challenges test for allergy directly they have been used as a putative gold standard to calculate the sensitivity and specificity of allergen-specific IgE or SPT [14,15]. However, challenge tests themselves have imperfect sensitivity and specificity for a variety of reasons, including the need for co-factors such as aspirin [16], exercise [17] or an exacerbation of asthma that may be absent under the circumstances of the challenge, failure to reach the threshold dose for reaction [18] and the effect of allergen preparation or excipient [19] on absorption or IgE-binding of the allergen. Double-blind food challenge tests also have the potential for false positive results depending on the probability of a placebo response P_p and the number of repeats of active and placebo challenge for each food. For example, if $P_p = 0.5$ and only one

pair of tests is performed, the probability of a false positive outcome (positive response to the test and negative to the placebo) is 0.25; at least three pairs of test and placebo at each dose would be required to reduce the probability of a false positive pattern to <0.05.

In conclusion, tests with imperfect sensitivity and specificity are at their most powerful when the prior probability is 0.5; this is good when identifying which of two allergens caused a reaction but poor if screening a population for allergy. The use of likelihood ratios and recognition that allergy test results around the optimal cut-off between positive and negative give no information about the probability of the patient being affected will enhance the value of these tests. Serum-specific IgE antibody measurement has poor specificity in those with atopic dermatitis and very high IgE and poor sensitivity in those with low total IgE. Challenge tests cannot be regarded as the gold standard unless a high enough dose is reached during the test, sufficient repeat tests are used to exclude false positives and all identifiable co-factors have been controlled.

Cell-based tests for diagnosis of allergy

There are certain clinical situations in which it is may only be possible to venture a tentative clinical diagnosis based on the patient's history alone, when skin prick and sIgE results are negative to all the probable causative allergens tested for. This includes the situation in which the production of IgE antibodies is restricted to a local site [20], and insufficient amounts are available to saturate local binding and catabolism and reach the general circulation to yield positive blood and skin prick tests. The failure to be able to identify a relevant sensitization may have a significant impact on the management of the patient even in low-risk clinical situations – for example, there may be uncertainty about whether to invest in (expensive) house-dust mite avoidance manoeuvres. Further steps in the investigative algorithms available in the laboratory might thus have significant value in the management of patients in various low and high clinical-risk situations such as:

- Perennial rhinitis to inform about house-dust mite or other sensitivity
- Asthma to inform as above
- Food allergy to confirm sensitization to a food and exonerate others
- Nut allergy to support the clinical diagnosis, to justify management
- Venom allergy: to confirm sensitization and the degree of cellular reactivity; to monitor if responding to desensitization immunotherapy; to determine when desensitization therapy should stop
- Drug allergy: to confirm reactivity to NSAID, antibiotic or other drugs; to investigate anaphylaxis to neuromuscular blocking drugs

A few cell-based tests have been developed for the above indications. All are relatively time-consuming, expensive and require more skilled laboratory input than skin prick and sIgE tests. Most studies of their use have thus far been on patients in high-risk situations, as these are often the most difficult to advise.

Another reason to favour the use of cell-based tests in some situations is their ability to give a summary measure of response in a functional assay that may reflect more realistically the situation *in vivo* where an individual's clinical response is influenced by many different input influences, many of which may be uncharacterized. IgE-mediated reactivity because of allergen cross-linkage of Fc ϵ R1-bound IgE antibodies on the surface membrane causes mast cell activation, but for a given level of sIgE, an individual patient's chance of developing symptoms on challenge with an allergen may vary from time to time. Mast cell responsiveness is enhanced by cytokines such as interleukin (IL)-13 [21] and influenced by variables such as dietary aspirin and other chemicals. It seems reasonable to envisage these multifactorial influences being integrated at the level of the mast cell.

Peripheral blood basophils also express Fc ϵ R1 and can thus act as an indicator cell for *in vitro* tests. Their activation can lead to release of preformed granules containing histamine and synthesis of a variety of mediators, including platelet-activating factor and leukotrienes, and assays are available for histamine and leukotriene release. CD63 is expressed on basophils [22], being a transmembrane protein expressed on the inner aspect of membrane-bound granules, which becomes expressed on the cell surface after fusion of the granule and cell surface membrane consequent upon activation. CD203c is expressed on the cell surface of basophils and its expression becomes upregulated upon activation [23]. Tests of basophil activation using these activation markers have yielded interesting research results, but their use in routine clinical diagnosis awaits standardization of assays, their sensitivity having proved thus far to be lower than that of SPT in tests for allergy to common allergens [24]. Other markers of basophil activation include the histamine released and leukotrienes synthesized upon activation (see below). Differences in the levels of histamine and leukotriene release, and CD63 and CD203c expression in different situations imply further levels of complexity that will need to be delineated before the full potential of these tests can be exploited. Such observations suggest that activation pathways can give rise to different sets of outcomes, implying that the analysis of a series of different cellular responses to a panel of stimuli might, in due course, be most informative.

Histamine release assays

The assay of the serum histamine concentration has, in the past, been used to confirm that anaphylaxis may have been

caused by mast cell activation. It is no longer used as it has been superseded by assay for serum tryptase (see below), which is more stable in serum. Histamine is a very labile molecule with a very short half-life in blood, thus making the collection, handling and transport of specimens logistically unworkable. Histamine release from sensitized basophils in peripheral blood has been used as one summary measure of the tendency of those basophils to become activated by an allergen. Assays such as the Immunoscreen histamine assay involve the release from whole blood of histamine after adding a stimulus. An acetylation agent is then added to the whole blood and the acetylated histamine product assayed by enzyme-linked immunosorbent assay (ELISA) immediately afterwards. This two-stage assay has given reliable results in experienced hands, but is cumbersome and unforgiving. The results are expressed as the histamine released as a percentage of the total histamine present in the cells in the whole blood sample (estimated by freezing an aliquot to release all the histamine from the cells present).

Major revisions in assay technology since the 1960s [25] have improved allergen quality, and variation in patient-group selection and differences in the cut-offs used in different studies make historical comparison of them difficult. Analytical improvements have not, however, led to improvements in sensitivity and specificity. The best-reported results have been on children with allergy to cat, dog or house-dust mite confirmed on bronchial provocation testing, among whom the histamine release assay had a sensitivity of 90% and specificity of 78% ($n = 81$) [26]. Some more recent studies have shown sensitivity to be as low as 38% [27] and 53% with specificity 82% [28].

Cellular antigen stimulation test assays

Basophils synthesize sulphidoleukotrienes (sLT) *de novo* from membrane lipids following some stimuli [29].

The Bühlmann cellular antigen stimulation test (CAST) requires stimulation of separated peripheral blood leucocytes (after dextran sedimentation of red blood cells) in the presence of IL-3. The sLT released into the cell suspension is then assayed by ELISA, which measures the sum of LTC $_4$, LTD $_4$ and LTE $_4$ (formerly collectively called slow reactive substance of anaphylaxis). The sensitivities and specificities reported for CAST assays vary significantly from one allergen to another. For hymenoptera venom they have both been > 90% [30], for *Dermatophagoides farinae* 71% and 87% [31], and for drug allergy 34% and 86% [32].

Logistics and economics dictate that assay methods incorporating analyte ELISA determinations after cellular stimulation can be performed realistically only in laboratories with a reasonably high demand for these tests. For the specialist indications for which they are most likely to be helpful, most laboratories may find flow cytometric methods to be more economical and convenient.

Basophil activation tests

The value of analysing basophil activation via expression of CD63 and CD203c by flow cytometry is currently being established. It is possible in future that other activation markers may prove as or more useful – for example, on activation basophils show a rapid increase in the expression of phosphorylated p38 mitogen-activated 3 kinase which correlates closely with increased CD63 expression [33]. The strategies used to identify and gate peripheral blood basophils have changed over the years. Initially they were identified on flow cytometry by high side-scatter, CD45 and IgE expression. More recently, expression of the IL-3 receptor (CD123) and the prostaglandin D2 receptor (CRTH2) have been used, and latterly simply a combination of side-scatter and expression of the eotaxin 1 and 2 receptor, CCR3 [27]. The anticoagulants used, the cell manipulations and other protocol details have major impacts on assay results, and require standardization. Other inherent assay difficulties include:

- Basophils are comparatively rare events in peripheral blood, and so even with modern flow cytometers with a high event-count rate, the acquisition times are long, it sometimes being necessary to collect events within a sample for up to 10–15 min.
- The assays have so far required analysis of shift in dose-response curves rather than single-dose evaluations, to ensure rigorous determination of multi-factorial matters. This adds to the labour and cost involved.
- If basophils are cells in transit from bone marrow to tissues rather than a phenotypically and functionally homogeneous circulating counterpart of tissue mast cells (this remains uncertain), they may display a spectrum of maturity from early entrants to the peripheral blood pool to mature cells almost ready to enter tissues to differentiate further there. Thus individual basophils may display different responses to various stimuli depending on their maturity. The spectrum of responses might also vary from one individual to another, or within an individual under different circumstances (e.g. diet). Interpretation of results of assays performed on a cell-by-cell basis might thus be very difficult, until such time as we have more knowledge of the phenotypic markers and functional differences that distinguish relevant basophil subpopulations in order to enable appropriate scrutiny of those that are the most informative for particular clinical circumstances.

The results reported for the use of basophil activation tests (BAT) in studies of patients with wasp venom allergy show sensitivities and specificities of up to 85–90%, comparable with skin prick and sIgE tests [34]. Sensitivities and specificities for the diagnosis of latex allergy have reportedly exceeded 90% [35], and those for the identification of neuromuscular blocking drug-induced anaphylaxis of 79 and 100% respectively [36].

The need to standardize these flow cytometric assays by applying them in the study of well-defined patient groups with allergy has led to the formation of an European multi-centre collaborative group (EuroBAT), the inaugural meeting of which was held recently and can be viewed online (<http://www.pharmaimage.tv>).

Other tests of utility in allergy performed in the immunology laboratory

Mast cell tryptase

Preformed mast cell mediators include histamine, proteoglycans and the neutral proteases tryptase and chymase. Determination of serum mast cell tryptase concentration is useful in two settings – to determine whether or not anaphylaxis has occurred (particularly in the unconscious patient, e.g. experiencing anaphylaxis during anaesthesia), and assisting in the diagnosis of mastocytosis syndromes [37]. The tryptase content of basophils and mast cells varies considerably. Basophils contain at most 0.04 pg of tryptase, whereas mast cell tumour (MCT) type mast cells in lung contain ~10 pg and MCTC in skin and gut contain as much as 35 pg/cell. Schwartz and colleagues determined that significant quantities of tryptase (>20 ng/ml) are released by mast cells into the circulation during anaphylaxis or in mastocytosis, but not in myocardial infarction or sepsis [38]. The same group determined the time-course of tryptase release during anaphylaxis [39], and demonstrated a serum peak concentration around 2 h after the onset of anaphylaxis. Tryptase is stable in separated serum samples, and analysis of serial samples taken at the time of incident, at 2 h and 24 h after onset of anaphylaxis, can be very helpful [40]. Recent work has shown that there may be a significant circadian variation in serum tryptase levels which should be borne in mind when analysing serial samples [41]. Serum tryptase determination is also essential in patients with skin/IgE test-negative anaphylactoid reactions to insect venom, as there is a significant possibility of undiagnosed mastocytosis in these patients [42]. However, not all anaphylactic reactions cause a rise in tryptase, and the rise is not always above the upper limit of normal: greater sensitivity can be achieved by using the percentage change rather than absolute level [43].

Serum complement assays

Patients with non-allergic angioedema constitute a high proportion of those referred to allergy clinics, and very few of these may have underlying hereditary angioedema. A low serum concentration of C4 in the presence of normal C3 has been used as a screen for C1-inhibitor deficiency [44], as most laboratories can assay C3 and C4 rapidly by nephelometry, but the value of this approach has been contested at borderline serum C4 concentrations [45,46]. Most would

accept that serum levels of C4 >0.15 g/l are extremely unlikely in hereditary angioedema but antigenic and functional assays of C1-inhibitor are important to exclude hereditary and acquired C1-inhibitor deficiency where there is any doubt. This subject is covered in more detail in a later article in this series.

Immunochemistry and autoantibodies

A number of investigations may be helpful in the work-up of selected patients with urticaria-like disorders. Schnitzler's syndrome, an unusual painful urticaria, is often associated with a paraprotein, which can convert to a B cell malignancy [47]. This is demonstrated using serum electrophoresis, and if necessary immunofixation. Urticarial vasculitis syndromes are an important differential diagnosis of chronic urticaria. Several forms exist, including those with SLE-like features, those with hypocomplementaemia and those with anti-C1q antibodies. Skin biopsy can be helpful in suspected cases [48] and determination of C3, C4 and the relevant autoantibodies should be discussed with the laboratory.

Anti-neutrophil cytoplasm (ANCA)-associated vasculitides such as Wegener's granulomatosis are an important differential diagnosis in patients with unusual presentations with rhinitis. The laboratory plays a key role in this test, as patient selection and consideration of the pretest probability of Wegener's is essential for the interpretation of ANCA results [49,50]. This is covered in more detail in a later article in this series.

Tests in allergic bronchopulmonary aspergillosis

Immunoglobulin E-mediated allergy to *Aspergillus* species can cause a 'brittle' form of asthma [51] called allergic bronchopulmonary aspergillosis, which is frequently underdiagnosed in asthmatic patients [52]. Older laboratory techniques using double-diffusion in agar (Ouchterlony) measured both IgE and IgG antibodies against aspergillus (known as 'precipitins') in a relatively insensitive, poorly reproducible qualitative method [53]. Current methods of assaying IgE antibodies against only *Aspergillus* use a more sensitive enzyme-linked immunoassay (Immunocap) with the ability to produce a reproducible, quantitative result [54]. The serum total IgE concentration is, however, correlated closely with the level of endobronchial allergic inflammation and thus serial total IgE assays constitute a good clinical guide to the amount of immunosuppression required with oral corticosteroids [55].

Diagnosis of humoral immunodeficiency

A significant proportion of rhinitis is not due to allergy. Infective causes can unusually be the result of antibody deficiency syndromes. Measurement of total IgG, IgA and IgM levels with electrophoresis to exclude a paraprotein along-

side, in selected cases, determination of pathogen-specific IgG levels (which have now superseded IgG subclass determinations for the investigation of more subtle forms of antibody deficiency syndrome) is of importance. Antibody responses to the capsular polysaccharide of *Streptococcus pneumoniae* are frequently impaired in specific polysaccharide antibody deficiency [56]. Determination of anti-pneumococcal antibodies requires clinical interpretation and, where necessary, measurement of pneumococcal strain-specific IgG levels [57].

Laboratory accreditation

Good laboratory practice mandates participation in external quality assessment (EQA) schemes that promote consensus on results regardless of the analytical method employed. For sIgE antibody assay the complexity and differing methods of manufacture of allergens represent a major source of inter-laboratory variation, particularly as some laboratories still manufacture allergen reagents in-house. The need for consistent quality is essential for any laboratory undertaking allergy testing and EQA in a consultant-led service are essential features of approval by Clinical Pathology Accreditation, the major laboratory accreditation organization in the United Kingdom which assesses eight major areas or standards in laboratory inspection by external peer-reviewed assessment:

- (A) Organization and quality management system
- (B) Personnel
- (C) Premises and environment
- (D) Equipment, information systems and materials
- (E) Organization and design of the EQA scheme
- (F) Operation of the EQA scheme
- (G) Communication with participants
- (H) Evaluation and improvement

The results of the EQA testing are displayed prominently in the laboratory and it is the right of clinicians using the laboratory to review these so that they are assured of the quality of the service being offered to their patients.

Future developments in laboratory testing for allergy

Any developments in laboratory testing for allergy must add in some way to the existing methodologies employed. Many molecules such as cytokines and chemokines play a crucial part in allergy, some of which have been described. Evaluation of the utility of assays for routine clinical assessment of these will involve significant work, but assays of potentially great relevance may arise. One promising example is the use of the thymus and activation-regulated chemokine in the assessment and monitoring of eczema [58]; another is the development of additional tests of mast cell degranulation,

perhaps combined with better analysis of serial measurements to use in a predictive fashion [43].

The role of immunoelectrophoresis and immunoblotting techniques to identify the molecular mass and other characteristics of allergens binding to a patient's IgE are, at present, research tools and are likely to remain so. A number of studies using native and recombinant allergens have shown the utility of microarrays in allergy testing [59]. This involves robotically spotting tiny amounts of different allergens onto coated glass slides, making it possible to test literally thousands of allergens in triplicate at once. This approach is likely to have advantages in terms of the large number of tests which can be performed simultaneously on small volumes of blood, increased automation, standardization and, for the first time, the potential to determine patterns of reactivity. Anticipated difficulties include generation of conflicting data, false positive results, inexplicable cross-reactivity and the relationship of these to skin prick test and other results.

In future it may be possible using this technology to assay linear, conformational, native and recombinant epitopes to reveal in detail an individual's response to a wide range of allergens and potentially any changes occurring over time or with treatment.

References

- Sewell WAC, Dore PC. Premature testing of allergen-specific IgE post-anaphylaxis may cause false negative results. *J Allergy Clin Immunol* 2004; **113**:S241.
- Bennich H, Ishizaka K, Ishizaka T, Johansson SG. A comparative antigenic study of gamma E-globulin and myeloma-IgND. *J Immunol* 1969; **102**:826–31.
- Ceska M, Lundkvist U. A new and simple radioimmunoassay method for the determination of IgE. *Immunochemistry* 1972; **9**:1021–30.
- Wide L, Bennich H, Johansson SG. Diagnosis of allergy by an *in-vitro* test for allergen antibodies. *Lancet* 1967; **2**:1105–7.
- Ewan PW, Coote D. Evaluation of a capsulated hydrophilic carrier polymer (the ImmunoCAP) for measurement of specific IgE antibodies. *Allergy* 1990; **45**:22–9.
- Li TM, Chuang T, Tse S, Hovanec-Burns D, El Shami AS. Development and validation of a third generation allergen-specific IgE assay on the continuous random access IMMULITE 2000 analyzer. *Ann Clin Lab Sci* 2004; **34**:67–74.
- Petersen AB, Gudmann P, Milvang-Gronager P *et al.* Performance evaluation of a specific IgE assay developed for the ADVIA centaur immunoassay system. *Clin Biochem* 2004; **37**:882–92.
- Brown CR, Higgins KW, Frazer K *et al.* Simultaneous determination of total IgE and allergen-specific IgE in serum by the MAST chemiluminescent assay system. *Clin Chem* 1985; **31**:1500–5.
- El-Shanawany TM, Arnold H, Carne E *et al.* Survey of clinical allergy services provided by clinical immunologists in the UK. *J Clin Pathol* 2005; **58**:1283–90.
- Hamilton RG, Adkinson NF Jr. Clinical laboratory assessment of IgE-dependent hypersensitivity. *J Allergy Clin Immunol* 2003; **111** (Suppl 2):S687–701.
- Dorizzi RM, Senna G, Caputo M. Likelihood ratios and Fagan's nomogram: valuable but underrated tools for *in vitro* latex sensitization assessment. *Clin Chim Acta* 1999; **282**:175–83.
- Letter Fagan TJ. Nomogram for Bayes theorem. *N Engl J Med* 1975; **293**:257.
- Sampson HA. Utility of food-specific IgE concentrations in predicting symptomatic food allergy. *J Allergy Clin Immunol* 2001; **107**:891–6.
- Mehl A, Verstege A, Staden U *et al.* Utility of the ratio of food-specific IgE/total IgE in predicting symptomatic food allergy in children. *Allergy* 2005; **60**:1034–9.
- Hill DJ, Heine RG, Hosking CS. The diagnostic value of skin prick testing in children with food allergy. *Pediatr Allergy Immunol* 2004; **15**:435–41.
- Matsuo H, Morimoto K, Akaki T *et al.* Exercise and aspirin increase levels of circulating gliadin peptides in patients with wheat-dependent exercise-induced anaphylaxis. *Clin Exp Allergy* 2005; **35**:461–6.
- Aihara M, Miyazawa M, Osuna H *et al.* Food-dependent exercise-induced anaphylaxis: influence of concurrent aspirin administration on skin testing and provocation. *Br J Dermatol* 2002; **146**:466–72.
- van Odijk J, Ahlstedt S, Bengtsson U, Borres MP, Hulthen L. Double-blind placebo-controlled challenges for peanut allergy: the efficiency of blinding procedures and the allergenic activity of peanut availability in the recipes. *Allergy* 2005; **60**:602–5.
- Grimshaw KE, King RM, Nordlee JA, Hefle SL, Warner JO, Hourihane JO. Presentation of allergen in different food preparations affects the nature of the allergic reaction – a case series. *Clin Exp Allergy* 2003; **33**:1581–5.
- Huggins KG, Brostoff J. Local production of specific IgE antibodies in allergic-rhinitis patients with negative skin tests. *Lancet* 1975; **2**:148–50.
- Finkelman FD, Rothenberg ME, Brandt EB, Morris SC, Strait RT. Molecular mechanisms of anaphylaxis: lessons from studies with murine models. *J Allergy Clin Immunol* 2005; **115**:449–57; quiz 58.
- Sainte-Laudy J, Vallon C, Guerin JC. Analysis of membrane expression of the CD63 human basophil activation marker. Applications to allergologic diagnosis. *Allerg Immunol (Paris)* 1994; **26**:211–14.
- Boumiza R, Monneret G, Forissier MF *et al.* Marked improvement of the basophil activation test by detecting CD203c instead of CD63. *Clin Exp Allergy* 2003; **33**:259–65.
- Hamilton RG, Franklin Adkinson N Jr. *In vitro* assays for the diagnosis of IgE-mediated disorders. *J Allergy Clin Immunol* 2004; **114**:213–25; quiz 26.
- Lichtenstein LM, Norman PS, Connell JT. Comparison between skin-sensitizing antibody titers and leukocyte sensitivity measurements as an index of the severity of ragweed hay fever. *J Allergy* 1967; **40**:160–7.
- Ostergaard PA, Ebbesen F, Nolte H, Skov PS. Basophil histamine release in the diagnosis of house dust mite and dander allergy of asthmatic children. Comparison between prick test, RAST, basophil histamine release and bronchial provocation. *Allergy* 1990; **45**:231–5.
- Monneret G, Benoit Y, Debard AL, Gutowski MC, Topenot I, Bienvenu J. Monitoring of basophil activation using CD63 and CCR3 in allergy to muscle relaxant drugs. *Clin Immunol* 2002; **102**:192–9.
- Li TM, Kontis KJ, Rance F, Abbal M, Vuitton DA. Clinical evaluation of a new fully automated enzyme immunoassay for basophil

- histamine release in whole blood. *Inflamm Res* 2000; **49** (Suppl 1):S49–50.
- 29 Mustafa FB, Ng FS, Nguyen TH, Lim LH. Honeybee venom secretory phospholipase A2 induces leukotriene production but not histamine release from human basophils. *Clin Exp Immunol* 2008; **151**:94–100.
 - 30 Royer B, Harraga S, Meyer J-P, Lustgarten V, Adessi B, Kantelip J-P, Da V. Valeur diagnostique des prick-tests, des IgE spécifiques et du test de libération d'histamine pour le diagnostic de routine d'allergie aux hyménoptères [Diagnostic value of skin-prick tests, specific IgE and histamine release for routine diagnosis of hymenoptera allergy]. *Rev Française Allergol Immunol Clin* 2004; **44**:144–50.
 - 31 Rossi RE, Monasterolo G, Operti D. A comparative study of the tryptase release test and the cellular allergen stimulation test (CAST) in mite sensitive patients. *Clin Exp Allergy* 1998; **28**:752–7.
 - 32 Lebel B, Messaad D, Kvedariene V, Rongier M, Bousquet J, Demoly P. Cysteinyl-leukotriene release test (CAST) in the diagnosis of immediate drug reactions. *Allergy* 2001; **56**:688–92.
 - 33 Ebo DG, Dombrecht EJ, Bridts CH, Aerts NE, de Clerck LS, Stevens WJ. Combined analysis of intracellular signalling and immunophenotype of human peripheral blood basophils by flow cytometry: a proof of concept. *Clin Exp Allergy* 2007; **37**:1668–75.
 - 34 Sturm GJ, Bohm E, Trummer M, Weiglhofer I, Heinemann A, Aberer W. The CD63 basophil activation test in Hymenoptera venom allergy: a prospective study. *Allergy* 2004; **59**:1110–17.
 - 35 Sanz ML, Gamboa PM, Garcia-Aviles C *et al.* Flow-cytometric cellular allergen stimulation test in latex allergy. *Int Arch Allergy Immunol* 2003; **130**:33–9.
 - 36 Sudheer PS, Hall JE, Read GF, Rowbottom AW, Williams PE. Flow cytometric investigation of peri-anaesthetic anaphylaxis using CD63 and CD203c. *Anaesthesia* 2005; **60**:251–6.
 - 37 Hennessy B, Giles F, Cortes J *et al.* Management of patients with systemic mastocytosis: review of M. D. Anderson Cancer Center experience. *Am J Hematol* 2004; **77**:209–14.
 - 38 Schwartz LB, Metcalfe DD, Miller JS, Earl H, Sullivan T. Tryptase levels as an indicator of mast-cell activation in systemic anaphylaxis and mastocytosis. *N Engl J Med* 1987; **316**:1622–6.
 - 39 Schwartz LB, Yunginger JW, Miller J, Bokhari R, Dull D. Time course of appearance and disappearance of human mast cell tryptase in the circulation after anaphylaxis. *J Clin Invest* 1989; **83**:1551–5.
 - 40 Brown SG. Cardiovascular aspects of anaphylaxis: implications for treatment and diagnosis. *Curr Opin Allergy Clin Immunol* 2005; **5**:359–64.
 - 41 Dugas-Breit S, Przybilla B, Schopf P, Rueff F. Possible circadian variation of serum mast cell tryptase concentration. *Allergy* 2005; **60**:689–92.
 - 42 Golden DB, Tracy JM, Freeman TM, Hoffman DR. Negative venom skin test results in patients with histories of systemic reaction to a sting. *J Allergy Clin Immunol* 2003; **112**:495–8.
 - 43 Brown SG, Blackman KE, Hedde RJ. Can serum mast cell tryptase help diagnose anaphylaxis? *Emerg Med Australas* 2004; **16**:120–4.
 - 44 Gompels MM, Lock RJ, Morgan JE, Osborne J, Brown A, Virgo PF. A multicentre evaluation of the diagnostic efficiency of serological investigations for C1 inhibitor deficiency. *J Clin Pathol* 2002; **55**:145–7.
 - 45 Karim Y, Griffiths H, Deacock S. Normal complement C4 values do not exclude hereditary angioedema. *J Clin Pathol* 2004; **57**:213–14.
 - 46 Tarzi MD, Hickey A, Forster T, Mohammadi M, Longhurst HJ. An evaluation of tests used for the diagnosis and monitoring of C1 inhibitor deficiency: normal serum C4 does not exclude hereditary angio-oedema. *Clin Exp Immunol* 2007; **149**:513–16.
 - 47 Lim W, Shumak KH, Reis M *et al.* Malignant evolution of Schnitzler's syndrome – chronic urticaria and IgM monoclonal gammopathy: report of a new case and review of the literature. *Leuk Lymph* 2002; **43**:181–6.
 - 48 Davis MD, Brewer JD. Urticarial vasculitis and hypocomplementemic urticarial vasculitis syndrome. *Immunol Allergy Clin North Am* 2004; **24**:183–213;vi.
 - 49 Savige J, Gillis D, Benson E *et al.* International Consensus Statement on Testing and Reporting of Antineutrophil Cytoplasmic Antibodies (ANCA). *Am J Clin Pathol* 1999; **111**:507–13.
 - 50 Schmitt WH, van der Woude FJ. Clinical applications of antineutrophil cytoplasmic antibody testing. *Curr Opin Rheumatol* 2004; **16**:9–17.
 - 51 Zureik M, Neukirch C, Leynaert B, Liard R, Bousquet J, Neukirch F. Sensitisation to airborne moulds and severity of asthma: cross sectional study from European Community respiratory health survey. *BMJ* 2002; **325**:411–14.
 - 52 Maurya V, Guhani HC, Sarma PU, Madan T, Shah A. Sensitization to *Aspergillus* antigens and occurrence of allergic bronchopulmonary aspergillosis in patients with asthma. *Chest* 2005; **127**:1252–9.
 - 53 Aberer W, Woltsche M, Woltsche-Kahr I, Kranke B. IgG antibodies typical for extrinsic allergic alveolitis – an inter-laboratory quality assessment. *Eur J Med Res* 2001; **6**:498–504.
 - 54 Lopata AL, Schinkel M, Potter PC *et al.* Qualitative and quantitative evaluation of bird-specific IgG antibodies. *Int Arch Allergy Immunol* 2004; **134**:173–8.
 - 55 Imbeau SA, Nichols D, Flaherty D, Dickie H, Reed C. Relationships between prednisone therapy, disease activity, and the total serum IgE level in allergic bronchopulmonary aspergillosis. *J Allergy Clin Immunol* 1978; **62**:91–5.
 - 56 Vendrell M, de Gracia J, Rodrigo MJ *et al.* Antibody production deficiency with normal IgG levels in bronchiectasis of unknown etiology. *Chest* 2005; **127**:197–204.
 - 57 Balmer P, North J, Baxter D *et al.* Measurement and interpretation of pneumococcal IgG levels for clinical management. *Clin Exp Immunol* 2003; **133**:364–9.
 - 58 Song TW, Sohn MH, Kim ES, Kim KW, Kim KE. Increased serum thymus and activation-regulated chemokine and cutaneous T cell-attracting chemokine levels in children with atopic dermatitis. *Clin Exp Allergy* 2006; **36**:346–51.
 - 59 Bacarese-Hamilton T, Gray J, Ardizzoni A, Crisanti A. Allergen microarrays. *Methods Mol Med* 2005; **114**:195–207.