

Measuring Allergen-Specific IgE

Where Have We Been and Where Are We Going?

M. Michael Glovsky

Summary

About 40 yr ago, two groups of investigators identified a new class of immunoglobulins, IgE. By exchanging their results and reagents, they proved that the immunoglobulin responsible for immediate hypersensitivity was IgE. From that day forward the science of allergy was greatly advanced. Within a few years of the IgE discovery, an assay for IgE was developed. This test was named the radio allergosorbent test. The specific IgE testing methodology has matured in the last four decades. Different means of detecting IgE bound to allergen is the subject of this review. We have included methods for measuring specific IgE using the ImmunoCAP 1000 instrument. The methodology for measuring basophile histamine release is also detailed in this chapter.

Key Words: Allergy; RAST; immunoassay; allergen-specific IgE; ImmunoCAP.

1. Introduction

IgE, the immunoglobulin isotype related to immediate hypersensitivity (allergy), was identified by two groups of investigators in 1966 and 1967. The Ishizakas, in Denver, extracted a new class of immunoglobulins (not IgG, IgA, IgM, or IgD) from reaginic sera of individuals highly allergic to ragweed pollen (1). When the isolated immunoglobulin was passively injected into human skin and the skin was challenged with ragweed extract, a wheal and flare reaction occurred. Within the same years Bennisch and Johanson in Sweden had identified a patient, N. D., with an unusual myeloma protein not identified by antisera to the known immunoglobulin isotypes (2). They called this myeloma protein Ig N. D. The two groups compared the myeloma protein with the isolated immunoglobulin and proved that the reagenic antibody and IgE N.D. were similar in structure, antibody reactivity, and function, and named the allergy antibody "IgE" (2).

From: *Methods in Molecular Biology*, vol. 378, *Monoclonal Antibodies: Methods and Protocols*
Edited by: M. Albitar © Humana Press Inc., Totowa, NJ

Diagnostic testing for allergy has progressed since the discovery of IgE. In 1967, Wide and colleagues introduced an in vitro test for specific allergens (3). They produced antibody to human IgE in rabbits and coupled specific allergen protein (such as ragweed or ryegrass extract) to cellulose discs. Next, they added sera of individuals allergic to ragweed, ryegrass, or other allergens to the specific allergen-coated cellulose discs. After washing the discs to remove unbound antibody, they added I^{125} labeled anti-IgE to the paper discs. The I^{125} that bound to the discs was analyzed to determine the quantity of specific IgE. This test was named the Radio Allergo Sorbent Test, or RAST (Fig. 1). Since its initial development, the RAST has been modified and automated. Other versions of the specific IgE test have been formulated, such as the MAST, AlaSTAT[®], and Hycor-EIA tests. All are based on similar methodologies. This review will focus on the ImmunoCAP–FEIA methodology, thought by some investigators to be the most accurate and best-validated in vitro specific IgE test available.

ImmunoCAP is a third-generation quantitative test developed by Pharmacia Diagnostics from the original RAST technology (4). Also included in this chapter is an overview of newer methods, including flow cytometry, upregulation of basophile receptors CD_{63} and CD_{203} , and silica chip evaluation of specific IgE using recombinant allergens.

2. Specific IgE Testing With ImmunoCAP

The third-generation Pharmacia ImmunoCAP-specific IgE technology uses an activated sponge-like matrix to absorb specific allergens. The tests are sandwich immunoassays using a flexible, hydrophilic carrier polymer enclosed in a capsule as the solid phase (the ImmunoCAP). Serum is added to the ImmunoCAP and incubated, and the cap tube is then washed. Enzyme-labeled anti-IgE is added to the cap, and after further washing the bound IgE is quantified using a fluorescent substrate. Results are reported quantitatively using a kU/L scale. The calibrator is IgE bound to anti-IgE caps using a six-point quantitative curve. Calibration ranges from <0.35 kU/L to >100 kU/L. Results can be reported by a second system using six classes (0, 1, 2, 3, 4, 5, and 6) (Table 1).

CAP testing has been highly automated on several instruments such as the AutoCAP 100, ImmunoCAP 250, and ImmunoCAP 1000; the numeric suffixes indicate the numbers of tubes that can be analyzed.

2.1. Method for Measurement of Specific IgE Using the Unicap 1000 Instrument

2.1.1. Analytical Principle—UniCAP Specific IgE

The UniCAP-specific IgE FEIA is an in vitro quantitative assay that measures the concentration of circulating allergen-specific IgE in human blood samples.

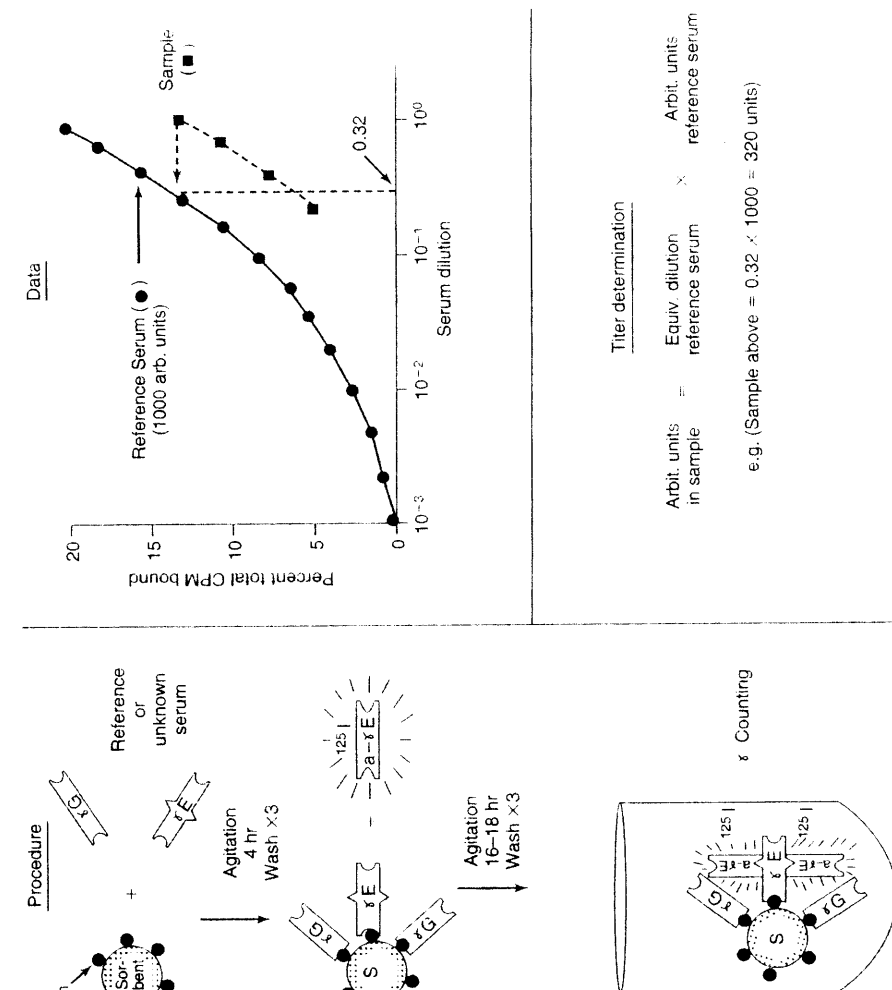


Fig. 1. Schematic of original radioallergosorbent test reported in 1967. In the first assay incubation, human antibodies of different isotypes (e.g., IgG, IgE) in reference and unknown sera bind to antigens coupled to the sorbent (allergosorbent). After isotonic buffer washes to remove unbound serum proteins, bound IgE antibody is detected with I^{125} -labeled rabbit anti-human IgE detection antibody. The percentage of total radioactivity in counts per minute (CPM) bound to the allergosorbent is then measured in a gamma counter and plotted against the serum dilution analyzed. The amount of specific IgE in reference and original sera is proportional to the amount of radioactivity bound. Interpolated units of antibody are corrected for sample dilution and reported as the number of arbitrary units of allergen-specific IgE in the original serum. (Reproduced with permission from ref. 18.)

Table 1
Relative Concentration of Specific IgE

Class	Relative activity	kU _A /L
Class 0	Undetectable	<0.35
Class 1	Low	0.35–0.70
Class 2	Moderate	0.71–3.50
Class 3	High	3.5–17.5
Class 4	Very high	17.6–50
Class 5	Very high	51–100
Class 6	Very high	>100

The calibration reflects total IgE bound and not specific IgE. Specific IgE to ryegrass, short ragweed, and cat extract would be ideal for use as a standard for each assay. However, such specific standards for over 400 assays would be technically impractical. For that reason, bound IgE is used as a relative calibrator for both specific and total IgE.

In principle, the allergen of interest, covalently coupled to ImmunoCAP, captures the specific IgE in the patient specimen. Nonspecific IgE is washed away and enzyme-labeled antibodies against IgE are then added to form a complex. After incubation, unbound enzyme-anti-IgE is washed away and the bound complex is incubated with a developing agent. The reaction is then stopped and the fluorescence of the eluate is measured. To evaluate the test results, the response units for patient samples are compared directly to the response of the calibrators.

The IgE calibrators are traceable to the Second International Reference Preparation 75/502 of Human Serum Immunoglobulin E from the World Health Organization (WHO). The UniCAP 1000 Instrument calculates the concentration in kU/L and the instrument software automatically calculates the concentration.

2.1.2. Equipment and Supplies

1. UniCAP 1000 instrument (cat. no. 12-3800-01).
2. UniCAP 1000 pipet tips in racks (cat. no. 12-3805-04).
3. Solid waste container bags (cat. no. 12-3807-36).

2.1.3. Reagent Summary, Preparation, and Storage (see **Note 1**)

All reagents are manufactured by Pharmacia and Upjohn Diagnostics, Uppsala, Sweden. **Note:** date and initial all reagents upon opening. Each container should be labeled with substance name, lot number, date of preparation, expiration date, and any special storage instructions.

1. Anti-IgE ImmunoCAP (cat. no. 14-4417-01). Anti-IgE Mouse monoclonal antibodies. 16 ImmunoCAP/carrier. Store at 2–8°C.

2. Allergen-specific ImmunoCAP (refer to catalog for specific allergen product number). Each ImmunoCAP is specific for detecting IgE antibodies against one or multiple types of allergen. Store at 2–8°C.
3. UniCAP-specific IgE conjugate 400 (cat. no. 10-9310-02). β -galactosidase-anti-IgE (mouse monoclonal antibodies) approx 1 μ g/mL. Color-coded blue. Six vials, 20.5 mL/vial. Store at 2–8°C.
4. UniCAP-specific IgE calibrator strip (cat. no. 10-9386-01). Human IgE in buffer, concentrations 0.35, 0.70, 17.5, 50, and 100 kU/L (0.2 mL/vial), five strips, one curve per strip. Store at 2–8°C.
5. UniCAP-specific IgE positive control (cat. no. 10-9446-01). Prepared from selected pooled human serum and contains IgE antibodies to a number of different allergens. Each kit contains six bottles with four determinations per bottle (0.3 mL each). Store at 2–8°C.
6. UniCAP-specific IgE negative control (cat. no. 10-9445-01). Prepared from human serum. Each kit contains six bottles with four determinations per bottle (0.3 mL each). Store at 2–8°C.
7. UniCAP development solutions (cat. no. 10-9439-01): 4-methylumbelliferyl- β -D-galactosidase 0.01%. Six bottles with 1200 determinations per bottle (64 mL per bottle). Store at 2–8°C.
8. UniCAP stop solutions (cat. no. 34-2271-51): sodium carbonate 4%, one bottle with 1200 determinations per bottle (850 mL per bottle). Store at 2–8°C.
9. UniCAP/Pharmacia CAP System washing solution set (cat. no. 10-9202-01). Set contains two bottles each of washing solution additive (86 mL) and washing solution concentrate (400 mL). Store at 2–8°C. To prepare for use, reconstitute using 10 L of distilled water per kit. Shake gently to mix. Prepared washing solution may be kept on the instrument. The washing solution bottle must be emptied and rinsed with distilled or ionized water. Water during weekly maintenance. Store at room temperature.

2.1.4. Testing of New Lots of Reagents

New lots of reagents and kits must be tested for performance, and the results of such testing recorded, before being placed in service. Include patient samples in parallel. To be acceptable, the new lot must perform the same as the old lot. New vendor shipments of in-use lots are also tested prior to use.

2.1.5. Calibrators and Standards

UniCAP-specific IgE calibrator strip (cat. no. 10-938601): human IgE in buffer, concentrations 0.35, 0.70, 3.50, 17.5, 50, and 100 kU/L. The IgE calibrators are traceable to the Second International Reference Preparation 75/505 of human serum IgE from WHO. Date and initial all calibrators upon opening. Each *aliquot tube* should be labeled with (1) substance name, (2) lot number, (3) date of preparation, (4) expiration date, and (5) any special storage instructions. Store at 2–8°C.

3. Procedure

3.1. Calibration Procedure

A six-point reference curve is set up along with the patient samples each time, and results are calculated by comparing the fluorescence of the unknown to that of the reference. The UniCAP 1000 Instrument measures specific IgE concentrations in kU/L and the instrument software automatically calculates the concentration.

3.2. Procedure

Follow the manufacturer's instructions.

4. Calculations

All calculations are preformed by the UniCAP operator software. For complete details, refer to the UniCAP 1000 system manual.

4.1. Other Commercial IgE Tests

4.1.1. AlaSTAT

The AlaSTAT IgE assay (Diagnostic Products Corp., Los Angeles, CA) employs biotin-labeled allergen extracts that bind in the fluid phase to specific IgE (5). The biotin-IgE complexes are added to microtiter wells or plastic tubes where biotin-albumin is coated to the surface. Avidin is added to the solution and the IgE-biotin complexes are linked to the biotin solid phase. An enzyme-linked anti IgE is used to calibrate the specific IgE.

Quantitative IgE calibrators are used to assess specific IgE in the complexes and the results are calculated and classified as kU/L bound or with specific classes. The results of the AlaSTAT-specific IgE are similar, though not necessarily identical, to those of the ImmunoCAP IgE. Like the ImmunoCAP system, the AlaSTAT system has been automated.

The specific IgE bound in the AlaSTAT system depends on the individual extracts (such as cat dander, perennial ryegrass, and so on) that are coupled with biotin. The specific extract and or the coupling procedure may, by altering the binding of the allergen or the extract content of major allergens, give different values than the ImmunoCAP procedure.

4.2. Hycor Test

The Hycor EIA and the Hycor Turbo-MP RIA (Hycor Stratogene, Garden Grove, CA) methods for specific IgE detection are both FDA cleared (6). In these tests, allergens are linked to cellulose discs and serum IgE is tested after incubation with the allergen-coated discs. The Hycor-EIA uses a modified RAST scheme in which low-level specific IgE is recorded as positive. These low-level tests may be clinically unimportant. The Hycor test is similar to the

original RAST technology, but may be less well standardized owing to the purity of the extracts and the comparative validity of the specific IgE.

4.3. MAST Test

In a chemiluminescent system from Hitachi Chemical Diagnostics (formerly called MAST), the allergens are linked to a thread (7). After incubation with sera containing IgE antibodies, the threads are washed, incubated with anti IgE, washed again, and then developed with a chemiluminescent indicator. The test results are reported as specific classes, 0–6, and the assay is considered a qualitative rather than a quantitative test.

5. Cell- and Microarray-Based Technologies to Detect Allergen-Specific IgE

5.1. Basophile Histamine Release

Histamine release using leukocytes from atopic individuals has been studied for the last half century (8,9). Leukocytes are separated from heparinized blood to remove red blood cells, and the volume of the leukocyte preparation is restored to the initial blood volume. The specific allergen under study is added to the leukocyte preparation (containing 0.5–1.0% basophiles) as well as controls. After an appropriate incubation, the cells are separated from the supernatant. Released histamine is then determined by comparing lysed basophiles (100% histamine) to buffer and other controls. A good correlation (~90%) between histamine release and skin testing as well as specific IgE is usually found with pollen allergens. With venoms and B-lactam drugs, the sensitivity vs skin testing is approx 50% (10). This discordance may be related to false-positive skin test reactivity or secondary to relative nonsensitivity of separated basophiles. In any event, basophile histamine release is more time-consuming and difficult to perform than specific IgE immunoassays, but is a relevant confirmatory test for unclear allergen biologic activity.

5.1.1. Method for Basophil Histamine Release: Evaluation of Allergic Status

Basophil histamine release in response to allergens (e.g., antigen E or Amb a1, Fel d1) correlates well with the severity of clinical symptoms suffered by individuals who are allergic to these proteins. In fact, allergen sensitivity is related to the serum of specific IgE, with very low concentrations of allergen often causing basophil histamine release in vitro when allergen-specific IgE antibody levels are high. As a result, release of histamine from basophils in vitro can serve as a dependable and precise indicator of an individual's allergic status, with few false-positive results when compared with skin testing techniques. Also, because many dilutions of antigens can be readily tested for reactivity, the assay can be more quantitative than skin testing, which often relies on a somewhat less precise endpoint.

5.1.2. Histamine Release From Washed Human Leukocytes

5.1.2.1. MATERIALS

1. 50-mL Polypropylene centrifuge tubes (Corning Inc. Corning, NY; cat. no. 25330).
2. 12 × 75-mm Polystyrene test tubes.
3. 2-mL Auto analyzer cups.
4. 1,4-piperazinebis (ethane sulfonic)-buffered saline containing 0.003% human serum albumin, 0.1% D-glucose (PAG) buffer.
5. 1,4-piperazinebis (ethane sulfonic)-buffered saline containing 0.003% human serum albumin, 0.1% D-glucose 10-M CaCl_2 and 0.1 mM NaCl_2 (PAGCM) buffer.

5.1.2.2. METHODS

The techniques described herein are, for the most part, used when histamine is analyzed by automated fluorimetry.

1. Blood is drawn into a plastic syringe, preferably using a butterfly infusion set with a needle size reflecting the desired volume required for the assay. Usually 1 mL of blood per reaction tube will provide total histamine levels of approx 20 ng with some variation occurring among donors.
2. Whole blood is immediately transferred to disposable 50-mL polypropylene centrifuge tubes, each containing 12.5 mL dextran, 5.0 mL 0.1 M EDTA, and 375 mg dextrose, which allows proper mixing for up to 20 mL of blood.
3. After sitting for 60–90 min at room temperature (23–25°C), or until a sharp interface develops between the plasma and erythrocyte pellet, the leukocyte-rich plasma layer is carefully removed using a disposable plastic pipet and transferred to a clean tube. The plasma is then centrifuged for 8 min at 110g in a refrigerated centrifuge at 4°C.
4. The upper plasma is carefully decanted (or aspirated) and discarded. The leukocyte pellet is gently resuspended and approx 40 mL (or twice the amount of blood used) of cold PAG buffer and centrifuged as previously listed.
5. The centrifugation and washing done in this manner are sufficient to remove most of the platelet contamination. It is sometimes useful to do one or more washes in PAG containing EDTA (~4 mM) because this reagent chelates calcium and prevents platelet clumping. However, it is important that the final wash be done in the absence of EDTA because histamine release requires calcium, and residual EDTA may prevent the reaction cascade. After the final wash, the cell pellet is resuspended in PAGCM buffer.
6. Histamine release is performed in polystyrene test tubes (12 × 75 mm). Total reaction volumes usually range from 0.1 to 1.0 mL. In practice, most experiments are run in a total volume of 0.1 mL. Because the sample volume necessary for automated fluorimetry is 0.5–1.0 mL, reactions performed in smaller volumes are brought up to 1.0 mL with PAG buffer at the end of the reaction, prior to harvesting the supernatants for histamine analysis.
7. For simple histamine release experiments, 0.02 mL each of 5X concentrations of antigen, buffer, perchloric acid, or other reagents are added to test tubes and kept at 4°C.

8. The washed leukocytes in PAGCM buffer and the reaction tubes are then warmed separately by incubating them for 5 min in a 37°C water bath before adding 0.08 mL of the cell suspension to each tube.
9. The total histamine content (called “completes”) is obtained by lysing the cells in a duplicate set of reaction tubes using perchloric acid at a final concentration of 1.6%. It is also important that tubes containing only cells and buffer (blanks) be included in each experiment as a measure of the spontaneous release of histamine (usually less than 5% of the total).
10. All conditions are tested in duplicate. During the reaction, the tubes are mixed by vigorously shaking the test tube racks every 15 min. At the end of 45 min at 37°C, reaction tubes are removed from the water bath and 0.9 mL of PAG buffer is added to each tube.
11. The tubes are then immediately centrifuged for 2 min at 1000g. Alternatively, the tubes can be centrifuged at 150g for 10 min.
12. The cell-free supernatants are decanted into 2 mL auto analyzer cups and stored at 4°C in plastic racks (Elkay Products Inc., Shrewsbury, MA). To prevent evaporation, samples should be frozen at –20°C if histamine cannot be analyzed within 2 wk. Alternatively, histamine can be measured using a histamine immunoassay.

5.2. Histamine Autoanalyzer Method (11)

The fluorometric technique remains the most popular method of assessing histamine release and is capable of consistently producing accurate and sensitive measurements of this histamine. Automation of this technique by Siraganian allowed analysis of up to 30 samples per hour (11); with more modern machines, as many as 45–60 samples can be analyzed per hour. The procedure is based on the extraction of histamine and its coupling with *o*-phthalaldehyde at a highly alkaline pH to form a fluorescent product. Samples tested must be relatively free of protein and other interfering compounds such as histidine. This is achieved by first extracting histamine into *n*-butanol from a salt-saturated, alkalized solution before the condensation step with OPT, histamine is back-extracted into an aqueous solution of dilute HCl by adding heptane. The histamine–ophthaldehyde complex is stable at an acid pH, which increases the fluorescent intensity of the compound. The automated technique requires a sample volume of 0.6–1.0 mL and is capable of detecting histamine levels in the range of 0.5 to >100 ng/mL. Although the automated methodology for histamine measurement is preferable, similar results can be obtained manually with minor loss of sensitivity and precision. The assay is linear from 0.5 to 1000 ng/mL. The advantage of automated fluorimetry over other systems is its ability to rapidly process a large number of samples. However, this is the method of choice only when samples are obtained from studies using low-protein buffer systems such as the *in vitro* release of histamine from basophile or mast cell cultures.

5.2.1. Special Reagents and Equipment for Flow Analyzer of Histamine

1. RFA 300 Rapid Flow Analyzer for histamine measurement by fluorimetry (Astoria-Pacific International, Clackamas, OR).
2. Filtered reagents for fluorimetry.
 - a. 0.73 M Phosphoric acid.
 - b. 30% NaCl.
 - c. 5 N NaOH.
 - d. 1 N NaOH containing 1 mM EDTA.
 - e. 0.1 N HCl.
 - f. Brij-saline-EDTA: 0.17 M NaCl, 1.5 mM EDTA, 0.015% Brij-35 (Perstorp Analytical, Silver Spring, MD).
 - g. Heptane.
 - h. Butanol.
 - i. OPT solution: 50 mg of OPT (Sigma, St. Louis, MO) recrystallized in Ligoine solvent (Eastman Kodak Co., Rochester, NY), 1 mL of spectranalyzed methanol (Fisher Scientific, Pittsburgh, PA), and 0.5 M 99 mL of borate buffer.
3. Histamine standards (for fluorimetry).
 - a. Histamine dihydrochloride (molecular weight = 184.1).
 - b. Histamine (molecular weight = 111.0).
 - c. 1 mg/mL Histamine solution. (**Note:** 1 mg of histamine equals 1.66 mg of histamine dihydrochloride.)
 - d. Dilute the 1 mg/mL solution 1:1000 in 2% HClO₄ to give 1 µg/mL stock and store at -20°C in 6-mL aliquots.
 - e. 6 mL of 1 µg/mL histamine added to 294 mL of 2% perchloric acid = 20 ng/mg.
 - f. 50 mL of ~20 ng/mL standard added to 50 mL of 2% perchloric acid = 10 ng/mL.

5.2.2. Buffer for Basophil Histamine Release

1. 10X PIPES buffer, pH 7.4: 250 mM PIPES (Sigma), 1.10 M NaCl, and 50 mM KCl.
2. PAG buffer: 10% 10X PIPES, 0.003% human serum albumin (Calbiochem-Bering Corp., La Jolla, CA), and 0.1% D-glucose.
3. PAG-EDTA buffer: PAG containing 4 mM EDTA.
4. PAGCM buffer: PAG containing 1 mM CaCl₂ and 1 mM MgCl₂.
5. Isotonic Percoll: nine parts Percoll (Pharmacia, Piscataway, NJ) plus one part 10X PIPES.

5.3. Going With the Flow

In the last decade, basophiles have been found to upregulate cell surface markers (CD₄₅, CD₆₃, CD₆₉, and CD₂₀₃). CD₆₃ is a transmembrane marker of basophiles, mast cells, macrophages, and platelets. The flow cytometry assay measures upregulation of CD₆₃ concentration on basophiles after incubation of specific allergens (drug, food, or pollen) with heparinized blood supplemented with the cytokine IL-3. The relative upregulation of CD₆₃ on basophiles is then determined by flow histograms after stimulation with allergen, compared to

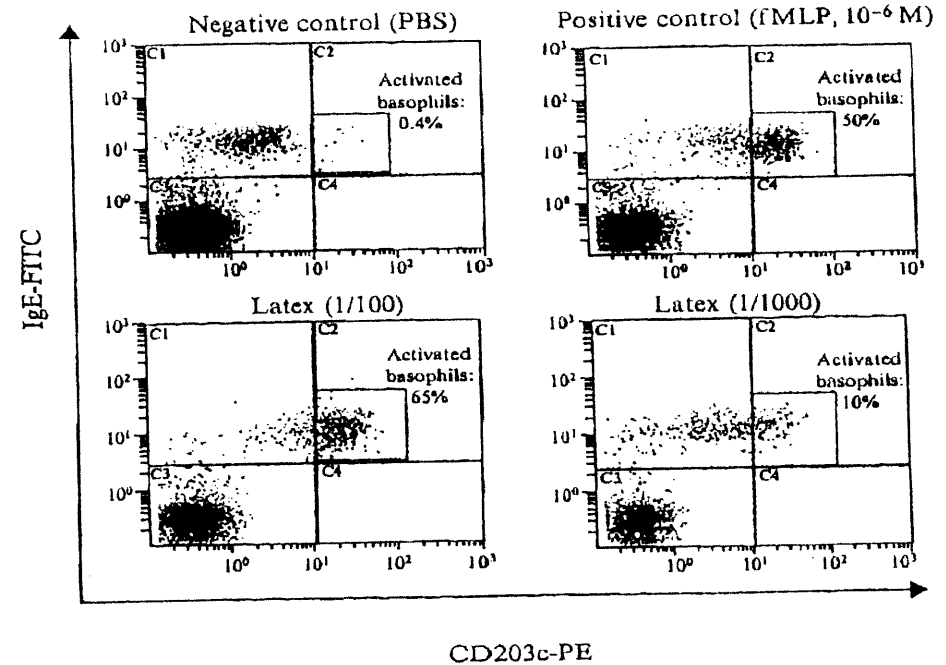


Fig. 2. Representative increased expression of CD203c after fMLP or allergen challenge. Gated cells are presented on the basis of CD203c-IgE staining: before stimulation (upper left dot-plot), after fMLP challenge (upper right), and after latex challenge at two different concentrations (lower dot-plots). (Reproduced with permission from *ref. 12.*)

appropriate controls. In one study of penicillin allergy, about 50% of the patients had a positive CD₆₃ increase compared to controls.

CD₂₀₃ is a neural cell surface biomarker found on IgE-bearing basophiles and mast cells. The advantage is that it is found primarily on basophiles. In a study of allergy to *Hymenoptera* (honeybee) venom, CD₂₀₃ was found to be upregulated from 4.2- to 13.5-fold in patients relative to normal controls. The CD₂₀₃ flow assay was found to confirm the presence of venom-specific IgE in 91% (20/22) of skin test positive patients who had clinical symptoms of honeybee sensitivity (12). In another study, latex-specific sensitivity was 63% and CD₂₀₃ upregulation was better than CD₆₃ (13) (Fig. 2).

A limitation of CD₂₀₃ studies with specific allergens is the viability of basophiles in human blood. Some investigators estimate that basophile function may last 24–36 h in heparinized blood. For transport of blood to a distant reference laboratory, the ability to receive the sample within a short time is a limiting factor. Partial separation of basophiles from other blood products could prolong the basophile viability. With certain classes of specific allergen reactivity

such as drug and food allergy, where skin testing may be inadequate, flow cytometry may provide valuable clues for patients sensitive to these allergens.

5.4. Microarray-Bound Allergens

The availability of recombinant allergens and recent advances in chip technologies have led to the development of new systems for the identification of specific IgE antibody. The ordered array with micrometer spacing could accommodate more than 1000 allergens and controls bound to the microchip surface (14).

Jahn-Schmid and colleagues in Vienna studied IgE binding to recombinant ryegrass and birch allergens linked to silica chips. In a study of 51 sera from atopic patients, the results obtained with microarray technology correlated well with those of the ImmunoCAP assay (15). An advantage of microarray methodology is the relatively small serum specimen required (<40 μ L). **Fig. 3** shows the relative activity of microarray vs skin testing and immunoblot techniques. Because the current validated ImmunoCAP tests have over 400 specific allergen extracts available and each extract may contain five or more major allergens, validating and comparing hundreds of recombinant allergens will await future comparative studies.

6. Conclusion

In the last 40 yr we have witnessed amazing advances in the science of identifying allergy antibodies and in the understanding of the immunology of allergic diseases. Since the discovery of IgE and the development of recombinant technologies, the in vivo and in vitro diagnosis of allergic reactivity has rapidly progressed. In vitro techniques are currently approaching in vivo (skin test) accuracy and time frames. Skin tests take 15–20 min from application of the allergen extract to final reading of the wheal and flare endpoint. Because skin tests are usually performed with nonstandardized extracts, the results may vary from lot to lot of the tests performed. Also, many of the tests, especially for environmental allergens such as house dust, molds, and animal dander, may show positive wheal swelling and negative IgE-specific results with ImmunoCAP. This discordance could be due to greater sensitivity of the skin tests or false-positive results secondary to an irritant response. In any event, the ImmunoCAP and other IgE tests are highly specific for the allergen tested. The specific IgE

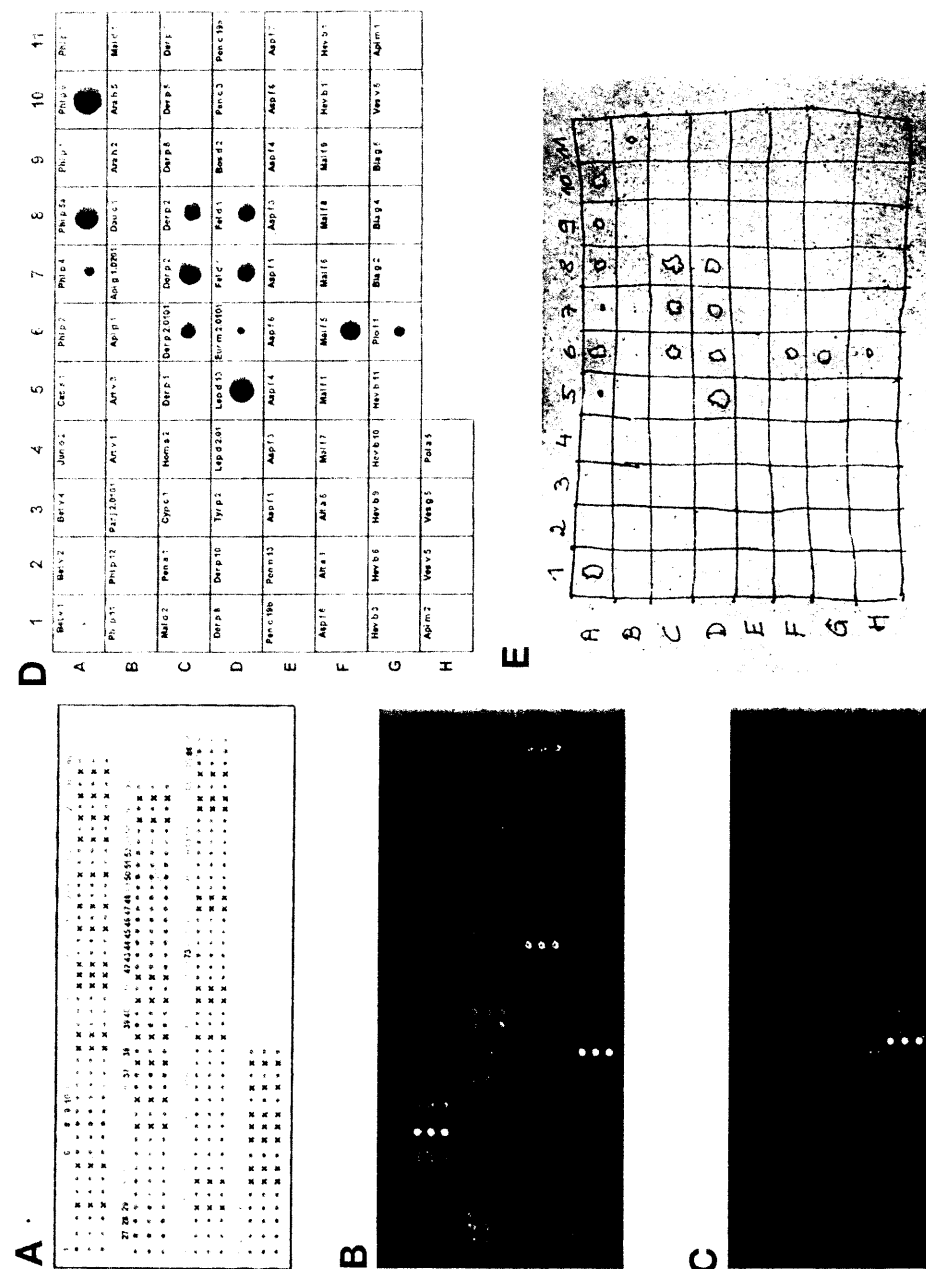


Fig. 3. Allergen microarray. (A) Scheme of environmental allergens spotted in triplicate in vertical columns. (B,C) Scanned images from a microarray chip-based IgE assay obtained with serum from an allergic (Fig. 2) (B) and nonallergic (Fig. 2) (C) individual. (D) Dot-blot IgE reactivity to the same allergens bound to nitrocellulose. (E) Skin reactivity to allergens and histamine (H6; wheal circled for visualization). (Reproduced with permission from ref. 14.)

tests may, however, be too sensitive: patients with class 3 or higher specific IgE (3.5–17.5 kU/L) may have food-related anaphylaxis (**16**) to important food allergens, whereas class 1 or 2 specific IgE (0.35–3.50 kU/L) to food allergens may not correlate with skin test positivity and or a history of food allergy (**16**). Thus, the relative clinical importance of skin testing, relevant clinical history and provocative tests, and specific IgE testing needs to be sorted out.

Current in vitro testing takes 2–4 h from the time serum is added to the analytical system until readout of the quantitative results. Patient time and discomfort can be minimized by a single venipuncture, and the time required to obtain results from in vitro tests may be similar to that for in vivo (skin) tests. It is not unreasonable to assume that allergy laboratory testing will take less time in the future. Laboratory testing is likely to be more accurate and nearly as quick, if not quicker than current skin test procedures. Currently, skin tests for drug allergy are not reliable because of the relative absence of purified drug metabolites that may be relevant in causing drug allergies. The lack of positive control subjects also limits the validity of drug testing. Flow cytometry may provide a highly specific and sensitive methodology to test relative drug sensitivity. In the future, a combination of biological testing (skin tests and or provocative tests) and specific IgE testing should provide important information for therapeutic intervention. However, the new technologies require careful validation to develop paradigms that encompass relevant clinical scenarios.

7. Notes

1. Date and initial all reagents upon opening. Each container should be labeled with substance name, lot number, date of preparation, expiration date, and any special storage instructions.

References

1. Ishizaka, K. and Ishizaka, T. (1967) Physicochemical properties of reaginic antibody. I. Association of reaginic activity with an immunoglobulin other than gamma A or gamma G globulin. *J. Allergy*. **37**, 169.
2. Bennich, H., Ishizaka, K., Ishizaka, T., and Johansson, S. G. O. (1969) A comparative antigenic study of gamma E globulin and myeloma IgND. *J. Immunol.* **102**, 826–831.
3. Wide, L., Bennich, H., and Johansson, S. G. O. (1967) Diagnosis of allergy by an in vitro tests for allergen-specific IgE antibodies. *Lancet* **2**, 1105–1107.
4. Paganelli, R., Ansoteugi, I. J., Sastre, J., et al. (1998) Specific IgE antibodies in the diagnosis of atopic disease. Clinical evaluation of a new in vitro test system, UniCAP, in six European allergy clinics. *Allergy* **53**, 763–768.
5. Ownby, D. R. and McCullough, J. (1994) Testing for latex allergy. *J. Clin. Immunoassay* **16**, 109–113.
6. Biagini, R. E., Krieg, E. F., Pinkerton, L. E., and Hamilton, R. G. (2001) Receiver

- serological assays for natural rubber latex-specific immunoglobulin E antibody. *Clin. Diagn. Lab. Immunol.* **8**, 1145–1149.
7. Hamilton, R. G. and Franklin Adkinson, N., Jr. (2004) In vitro assays for the diagnosis of IgE-mediated disorders. *J. Allergy Clin. Immunol.* **114**, 213–225.
8. Crockard, A. D. and Ennis, M. (2001) Basophil histamine release tests in the diagnosis of allergy and asthma. *Clin. Exp. Allergy* **31**, 345–350.
9. Nolte, H. (1993) The clinical utility of basophil histamine release. *Allergy Proc.* **14**, 251–254.
10. Maly, F. E., Marti-Wyss, S., Blumber, S., Cuhart-Stark, I., and Wuthrich, B. (1997) Mononuclear blood cell sulpholeukotriene generation in the presence of interleukin 3 and whole blood histamine release in honeybee and yellow jacket venom allergy. *J. Invest. Allergy Clin. Immunol.* **7**, 217–224.
11. Siraganian, R. P. (1974) An automated continuous-flow system for the extraction and fluorometric analysis of histamine. *Anal. Biochem.* **57**, 383–394.
12. Boumiza, R., Monneret, G., Forissier, M. F., et al. (2003) Marked improvement of the basophil activation test by detecting CD203c instead of CD63. *Clin. Exp. Allergy* **33**, 259–265.
13. Ebo, D. G., Hagendorens, M. M., Bridts, C. H., Schuerwegh, A. J., De Clerk, L. S., and Stevens, W. J. (2004) In vitro allergy diagnosis: should we follow the flow? *Clin. Exp. Allergy* **34**, 332–339.
14. Hiller, R., Laffer, S., Harwanegg, C., et al. (2002) Microarrayed allergen molecules: diagnostic gatekeepers for allergy treatment. *FASEB J.* **16**, 414–416.
15. Jahn-Schmid, B., Harwanegg, C., Hiller, R., et al. (2003) Allergen microarray: comparison of microarray using recombinant allergens with conventional diagnostic methods to detect allergen-specific serum immunoglobulin E. *Clin. Exp. Allergy* **33**, 1443–1449.
16. Sampson, H. A. and Ho, D. G. (1997) Relationship between food-specific IgE concentrations and the risk of positive food challenges in children and adolescents. *J. Allergy Clin. Immunol.* **100**, 444–451.
17. Lichtenstein, L., Norman, P., and Winkenwereder, W. (1968) Clinical and in vitro studies on the role of immunotherapy in ragweed hay fever. *Am. J. Med.* **44**, 514–524.
18. Adkinson, N. F., Jr. (1976) Measurement of Total Serum Immunoglobulin E and Allergen-Specific Immunoglobulin E Antibody. In: *Manual of clinical laboratory Immunology*, (Rose, N. R. and Friedman, H., eds.), American Society for Microbiology, Washington, DC, p. 590.