

In Vitro Assays for Immunoglobulin E

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Methodology, Indications, and Interpretation

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METHODOLOGY

Since 1967, when the first assays for serum immunoglobulin E (IgE) (1) and allergen-specific IgE antibodies in serum were described (2) many assay procedures have been tried. Only a few have become routine methods in clinical chemistry or immunology laboratories or in doctors' offices.

Serum IgE concentrations vary from less than one (10^{-11} M) to several ten thousand ng/mL, requiring extraordinary sensitivity and measuring range. The specific IgE antibody test requires a sensitivity down to the picogram range, as well as the ability to detect antibodies against hundreds of immunologically and chemically heterogeneous allergenic materials. From an analytical point of view, these situations are unique, and many of the assay procedures and reagent combinations suggested have had limitations in sensitivity, specificity, reproducibility, or other aspects determining their suitability for clinical routine application.

Immunoglobulin E Assays

Techniques

The earliest method for the measurement of total serum IgE was a competitive solid-phase radioimmunoassay (3). Such procedures involve one tracer-labeled reagent to increase the sensitivity and

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one reagent that can be removed from the liquid reaction mixture to facilitate the separation of bound tracer from free. In the first IgE assay, this latter reagent consisted of anti-IgE bound to Sephadex (Pharmacia Fine Chemicals AB, Uppsala, Sweden), a cross-linked dextran bead, which could be separated by centrifugation. Another approach was taken in the so-called double-antibody tests in which a second antibody was used to precipitate the immune complex formed by the tracer and the anti-IgE (4). Recently, the two approaches have been combined into a double-antibody, solid-phase assay (5,6). RIST

The solid-phase principle has also been developed into a completely different line of assay often referred to as sandwich immunoassays. In the paper radioimmunosorbent test (PRIST) (7) the reagents consist of anti-IgE chemically bound to cellulose and a tracer-labeled anti-IgE used to detect the amount IgE in the serum sample that has reacted with the solid-phase anti-IgE. The procedure requires no centrifugation and can easily be changed from radioactive labels to nonradioactive labels. It has been extensively used and frequently modified. One modification of the test has been used to measure levels down to about 0.1 µg/mL in culture supernatants (5,8). PRIST

Increasing demand for testing from physicians, together with a general trend toward nonradioactive reagents, has stimulated the development of tests that can be used by laboratories without radioimmunoassay facilities. The majority of the nonradioactive methods described are sandwich tests employing alternative labels, e.g., enzymes (9), but methods based on entirely different principles, such as particle agglutination (10) and affinity chromatography (11) have also been described.

Reagents

Anti-Immunoglobulin E. The majority of assays described so far have been based on polyclonal anti-IgE, often produced in rabbits to minimize the risk of interference by antibodies against animal γ -globulins in the human sample (12). Extreme care must be taken in purifying anti-IgE, especially if it is to be used in sensitive assays. Even minute traces of anti-IgG can render the test results invalid. In a study that compared several polyclonal anti-IgEs,

only the Phadebas IgE PRIST (Pharmacia Diagnostics AB, Uppsala, Sweden) material (rabbit anti-IgE raised against E myeloma ND) was free of anti-IgG activity (13). A preparation of anti-IgE raised against E myeloma PS was recently shown to overestimate the IgE concentration in serum (14). The epitope specificity is, of course, also important. Antibodies to the D ϵ 2 antigens (15) seem to give the highest sensitivity whereas antibodies to nonisotype-specific determinants, such as idiotype specificities and light chain-dependent determinants, are not suitable for this purpose.

Monoclonal antibodies have also been used. Only a few direct comparisons have been made between poly- and monoclonals in the same test system. The polyclonals were reported to give more sensitive assays whereas the monoclonals may give linear IgE dilution curves over a wider range (11,16). It must be emphasized that the introduction of monoclonal antibodies does not necessarily improve an assay, but that every reagent has to be judged on its own merits.

Solid Phase. Polysaccharides, like dextran, agarose, and cellulose, have advantages in terms of the capacity and reproducibility of binding, and the stability of the reagents permit long shelf life. Several other polymers have also been employed. Passive coating of glass (17) or plastic materials such as polystyrene (18) or polyvinylchloride (19,20) have been used with varying degrees of success in IgE assays. Limitations in reproducibility owing to variations in binding capacity, interference by serum factors, and limited measuring ranges are observations frequently made for microtiter plate assays (14,21-25), regardless of the procedure used for the binding of the anti-IgE to the solid phase. Also nitrocellulose sheets (26), magnetic polyacrylamide beads (27-29), and latex particles for agglutination have been applied.

Labels. Many attempts have been made to directly label antibodies with tracer atoms other than the commonly used radioisotopes. So far, only enzyme labels have been capable of emitting signals strong enough to permit measurement of very low concentrations with the detection systems presently available. Fluorescence labels have been reported but show limited sensitivity (30,31). β -Galactosidase (9), horseradish peroxidase (32,33), and alkaline phosphatase (23-34) are frequently used enzymes that can be combined with chromogenic (22,23,25,29,35,36), fluorogenic (29,34,37,38), and luminescent counterparts (33,39).

Evaluation and Standardization

Ever since the first international IgE reference preparation was released by the World Health Organization (WHO), it has been possible to calibrate measurements of IgE in various body fluids and express the concentration in kilounits/liter (1 kU is approx. 2.4 μ g) (40). However, it became evident relatively soon thereafter that non-IgE factors in the samples may influence various methods differently. The very first competitive immunoassay (RIST) was shown to give too high values in the range below 50 kU/L (41) and was also found to be unsuitable for measurements in certain secretions because of nonspecific interference (4). Sandwich immunoassays should be investigated also for size and position of the so-called high-dose hook effect (42), which might occur at unacceptably low concentrations as a result of inadequate test conditions.

The fact that recently released commercial kits for IgE quantitation have been reported to yield from 50 to 150% of the expected values over a wide range (6,38) emphasizes the importance of scrutinizing every new assay in terms of the validity of calibrator, reproducibility, true sensitivity and specificity, and correlation to reference method.

Autoantibodies to IgE have been found in serum of allergic patients. These are of the IgM (43) and IgG (44) type, both free and in complex (44). The antibody concentration is usually very low and only a small portion of the IgE seems to be complexed. Additionally, rheumatoid factors (45) and antinuclear antibodies (46) of the IgE type have been described. It is possible that in certain cases the presence of an anti-IgE can influence the results of an IgE determination.

Specific Immunoglobulin E Antibody Assays

Techniques

The radioallergosorbent test (RAST) described in 1967 (2) was a unique assay at that time. It provided a method for immunoglobulin class-specific measurements of antibodies against a practically unlimited range of antigens at a sensitivity level never reached before. The original procedure based on suspensions of allergen-Sephadex particles and radiolabeled anti-IgE has been improved

and modified (47-50), and various alternative reagents have been described. (Examples are given under *Reagents* below.) Reversed modifications of the RAST that utilize labeled allergens have been described (51-54).

All the assays and semiquantitative tests for allergen-specific IgE antibodies are solid-phase immunoassays. The use of crossed radioimmuno-electrophoresis has been suggested for this purpose (55). However, only antibodies to allergens recognized by the rabbit can be detected, and when detected they may be underestimated owing to blocking of the IgE-binding sites by the IgG antibodies used to trap the allergens (56). Tests for histamine release from naturally or passively sensitized basophils (57) or basophil degranulation tests (58) are mediator releasability tests rather than assays of specific IgE antibody.

Reagents

Anti-Immunoglobulin E. The comments made above for serum IgE assays are valid also for the specific antibody tests. Some of the monoclonal antibodies described (14,16,28,59,60) are reported to be comparable to the anti-IgE of the most widely used in vitro assay for IgE antibodies, the Phadebas RAST (Pharmacia Diagnostics AB), although others are less sensitive in detecting specific IgE antibodies.

Labels. The majority of test systems described during the last few years are enzyme immunoassays. β -Galactosidase was used in colorimetric (9,22,28,36) and fluorometric (37) assays, horseradish peroxidase in colorimetric (20,26,61,62) and luminescence (39) tests, glucose oxidase in colorimetric (27), and alkaline phosphatase in colorimetric (63,64) as well as fluorometric (65) systems. The enzymes are mostly conjugated directly with the anti-IgE, but a few investigators have utilized biotin-avidin bridges (20,62).

Allergens. It is obvious that the sensitivity and specificity of an IgE antibody assay are dependent on the quality of the allergen preparations used. Even minor variations in the methods for collection, cultivation, or storage of allergen source materials may cause significant changes in the content of allergenic components. Degradation and contamination are major risks, which have to be considered and avoided (Table 1). Differences in allergens have been

failed to show any obvious correlation between IgE antibody levels in nasal secretion and severity of rhinitis, suggesting that several other factors are involved (93).

Several studies performed during the last few years seem to suggest that also some of the IgE of tear fluids may be locally produced (94-97). Measurements of IgE and IgG antibodies in tears are said to be helpful in diagnosis of vernal conjunctivitis although local production does not appear to be limited to atopic eye disorders (98). In allergic conjunctivitis, apart from spring pollen allergy and in allergic rhinitis, the IgE in the tear fluid has been reported to be a result of leakage from the serum rather than of local production (99). Conflicting data on IgE in other secretions have been reported (109). IgE has been found in intestinal fluid (100), polyp fluid (101-103), bronchial lavage (104), pleural effusion (105), middle ear effusion (106), cerebrospinal fluid (107), and breast milk (4). Small amounts of an Fc"-like IgE fragment have been found in rectal washings (108). Recent studies using a particle agglutination-counting immunoassay (PACIA), in which all IgE material is transferred to Fc" fragments, a procedure likely to eliminate most interfering factors, essentially failed to detect significant levels of IgE in any secretions (109).

It must be emphasized that before any measurements are made in a sample from new sources, studies on recovery and parallelism have to be performed, covering the entire expected range of IgE concentrations. Positive and negative interferences of antibodies or other macromolecules cannot be excluded until proven irrelevant for the particular method that is to be used.

Interference in Radioallergosorbent Tests by Immunoglobulin G Antibodies

The studies of Zimmerman et al. (110) where the investigators tried to minimize the allergen concentration in a RAST test drew attention to the fact that RAST using a solid phase with inadequate concentrations of allergen is sensitive to interference by non-IgE antibodies. When cellulose powder was compared with the disks, it was found that interference was negligible in the disk based system owing to the higher antigen capacity of the disk (111). In only 2 of

96 sera from patients on ragweed immunotherapy could interference be documented. Similar statements were made by Nelson (112) who reported that his studies on follow-up of grass pollen allergen immunotherapy suggested that interference by blocking antibodies with the Phadebas RAST was minor and that the decline in RAST levels represented true declines in specific IgE antibody levels.

In some studies of possible interference (113,114), the investigators did not acknowledge the basic rule for the evaluation of test systems, i.e., follow the manufacturers' instructions for use. A majority of the patients selected for these studies did indeed have specific IgE levels outside the working range of the test, where quantitative information cannot be expected.

Hamilton and Adkinson (115) found the interference to be variable and unlikely to cause false-negative test results. In a recent study on Phadebas RAST pollen allergens, Squire et al. (116) found that the suppression of IgE antibody binding by IgG antibody was only 3%. Phadebas RAST was thus less affected by rising IgG antibody concentrations during allergen immunotherapy than was the skin test.

Our own studies on the effect of increasing concentrations of IgG antibodies on the measurement of low, medium, and high concentrations of IgE antibody within the measuring range of the Phadebas RAST showed no or only modest interference. In no case was the diagnostic value influenced by this phenomenon.

INDICATIONS AND INTERPRETATIONS

Serum Immunoglobulin E Reference Values in Adults

The use of a test for the identification of pathological cases is traditionally based on the establishment of a "normal" range for the specimen. In the case of IgE such studies on healthy individuals have been reported from various parts of the world since 1967. The upper limit of the normal range considered as the cutoff between normal and allergic cases by laboratories in different countries varies from 100 to over 700 kU/L. It is evident that the importance of serum IgE measurement as a part of an allergy investigation is

highly dependent on the establishment of the cutoff level—the higher it is the less likely it is that patients will be subject to adequate further investigations.

Improved measuring techniques, better statistical methods, but—above all—stricter clinical criteria have led to a dramatic drop in the adult reference level for serum IgE.

Recent studies of IgE levels in healthy nonatopic adults showed means well below 20 kU/L. If all smokers were left out, the values dropped to 10 kU/L, which is likely to be very close to the mean level of truly healthy adults (117–123). Nonatopic smokers have IgE levels about twice as high as nonatopic nonsmokers (120, 121, 124).

The age of the individuals in the study group influences the results, especially if the selection criteria have not been strict enough to permit exclusion of all allergic individuals (119). Environmental conditions, i.e., degree of stimulation by parasites and other IgE-stimulating agents, influence the mean levels as exemplified by rural and urban blacks in Gambia (125) and urban blacks in the United States (126).

Serum Immunoglobulin E Reference Value in Infants and Children

As seen with adults, a great variety of "normal" values from more or less well-defined population samples have been reported. They differ because of varying selection criteria and environmental differences. A few studies show the development of serum IgE from birth into puberty (123, 127–130). The values reported by Kjellman et al. (127), are somewhat lower than most of the others. This is because very strict selection criteria were applied. Aiming at a group representing the normal as closely as ever possible, those who developed allergic symptoms during an 18-month observation period after the test were excluded from the data.

The results from the study by Bhalla et al. (130) on American children have been found to be statistically indistinguishable from those of Kjellman et al. It was also found that log IgE vs log age (months) showed a linear relationship (Fig. 2). A linear regression analysis on the combined data from both studies, totaling 466 children, permitted calculation of the most probable serum IgE levels

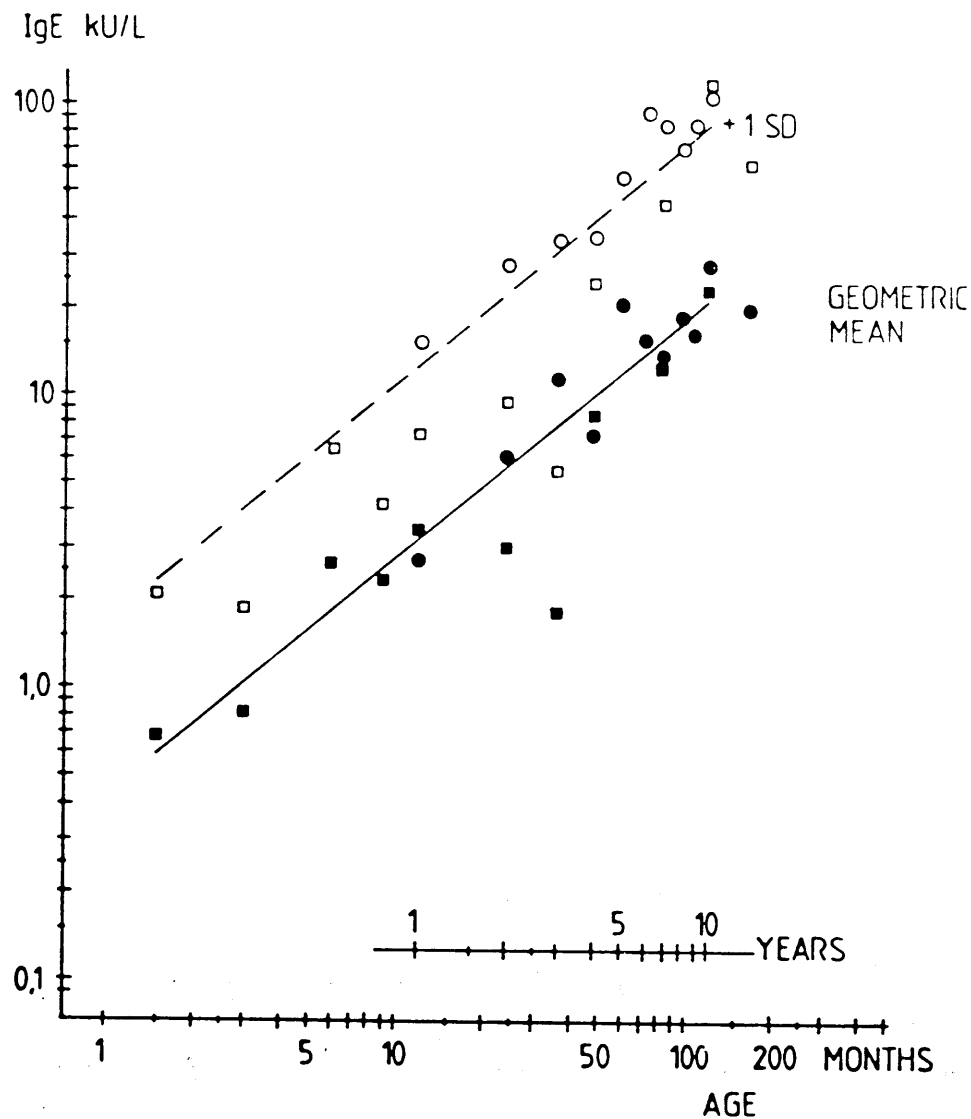


Fig. 2. Development of serum IgE with age during childhood. Data compiled from Kjellman et al. (127) and Bhalla et al. (130), $n = 466$.

(geometric mean and mean + 1 SD) for the ages from 6 wk to 10 y. Calculated data for the ages are shown in Table 2 (see p. 106). It is suggested that the mean + 1 SD, which at the age of 10 y

Table 2
Immunoglobulin Reference Levels in Childhood^a

Age	Geometric mean	+ 1 SD
6 w	0.59	2.33
3 mo	1.04	4.11
6	1.83	7.26
9	2.56	10.1
12	3.24	12.8
2 y	5.72	22.7
3	7.98	31.6
4	10.1	40.1
5	12.1	48.1
6	14.1	55.9
7	16.0	63.4
8	17.9	70.8
9	19.7	78.0
10	21.5	85.0

^aCalculated from the combined data of Kjellman et al. (127), and Bhalla et al. (130), based on 466 healthy children aged 6 w to 14 y.

approaches the 100 kU/L adapted by Zetterström and Johansson (131) as the upper cutoff for adults, is considered as "the borderline of normality" in the sense that higher values lead to continued investigations of the cause of the elevation: allergy, parasitosis, and so on.

Serum Immunoglobulin E Levels in Allergic Disease

Even in the first reports of IgE levels in asthma in 1967 (1), it was suggested that measurement of serum IgE could be valuable in discriminating between what at that time often was referred to as intrinsic and extrinsic disease. It was later shown that more sensitive methods were better suited for measurement of normal levels and had a higher discriminating power (117).

Not surprisingly, it was found that the IgE concentration was influenced by specific allergen stimulation. An increase was regularly found during the pollen season in pollen allergic individuals and in the early phase of immunotherapy (133). The more allergens a patient was sensitive to and the more symptoms he had, the higher the IgE level was (134).

IgE in Differential Diagnosis of Atopy-Nonatopy

In a basic study on the value of serum IgE measurements (131), the patient material comprised all patients referred to the county hospital for allergologic investigation during 1 y. Among them were a few who suffered from nonallergic diseases such as bronchitis and emphysema, but the rest all had allergic symptoms, mainly rhinitis or asthma. The patients with symptoms of allergic disease, totaling 445, were classified with regard to atopic status depending on results of skin tests, challenge tests, and RAST. Serum IgE levels were measured in 412 patients; 160 were classified as non-atopic, and 252 as atopic.

There was only a moderate overlap between the reference material and the atopic patients. Only 3 of 252 atopic patients had an IgE concentration lower than 20 kU/L whereas 63% of the healthy individuals had IgE values in this range. An IgE level of 100 or above was found in 2 of the 175 subjects in the healthy reference material and in the majority of the atopic patients.

To illustrate the relation between serum concentration and diagnostic classification, the group of 412 patients can be divided into three subgroups according to their IgE level: 70 patients (17%) had IgE levels below 25 kU/L, 125 patients (30%) had IgE levels between 25 and 100 kU/L, and 217 patients (53%) had IgE levels above 100 kU/L. The distribution of atopics and nonatopics in each group is shown in Table 3.

It is evident that the majority of the patients can either be correctly classified as nonatopic/atopic or recommended for further investigation based *only on the IgE level*. Only 11 patients (corresponding to 4.4% of the atopics or 2.7% of the patients) who had low-grade, mostly monovalent, pollen allergies would escape detection if an IgE above 25 kU/L was taken as the only criterion for extensive allergologic investigation. The combination of IgE with clinical history would certainly decrease this number. Similarly, the great majority of the patients of the group with IgE above 100 kU/L are definitely atopic and the offending allergens should be identified. The patients of this group were often sensitive to several types of allergens and a correlation between the number of allergen sensitivities and the IgE level was also demonstrated.

The measurement of total serum IgE provides essential information for the investigation of patients with suspected allergic diseases and is indicated in all cases where the clinical history is

Table 3
Distribution of Atopics and Non-Atopics in Patient Groups
with Low, Medium, and High Serum IgE

IgE concentration	<25 kU/L	25–100 kU/L	>100 kU/L
<i>n</i>	70	125	217
Percent atopic	16	57	78
Percent nonatopic	84	43	22

inconclusive. The study referred to above shows that about two-thirds of the patients can be classified as likely to be nonatopic (<25 kU/L) or atopic (>100 kU/L), the probability of correct classification is in both cases about 80%; further allergy testing of patients with levels below 25 kU/L is not indicated unless strongly supported by clinical history; the IgE level gives important guidance for further investigation regarding the range of allergens to be tested.

Serum Immunoglobulin in Prediction and Prevention of Atopic Allergy in Infants

Atopic allergy constitutes a major pediatric problem because of its high incidence and its serious consequences in adolescence. Preventive measures reduce the incidence of early-developing atopic symptoms but prevention should start at birth. This requires tools by which development of disease can be predicted.

Predisposition for atopic allergy is inherited and a reliable family history is helpful but often difficult to obtain. The child with two allergic parents is a high-risk child. However, the great majority of children have single or no heredity for allergy and a screening test is necessary to identify those at risk. The value of serum IgE measurements for the prediction of atopic allergy has been extensively studied (135–141). Healthy reference populations show levels well below 1 kU/L at birth (127,130).

Croner and Kjellman (139) found that 70% of children with a cord serum level higher than 1.3 kU/L developed allergy before 18 months of age. The corresponding figure for children with normal levels was 5%. Serum IgE measurement was found to be a more effective tool than family history (Table 4). A recent study by Kjell-

Table 4
Prediction of Allergic Disease in Neonates^a
(Before the Age of 18 m)

Predictive tool	Predictive value, percent	
	Positive	Negative
Family history	11	95
IgE measurement ^b	70	95

^aAdapted from Croner et al. (139).

^b>1.3 kU/L (Phadebas IgE PRIST).

man and Croner (141) shows that more than 90% of children having a long-lasting atopic disease during their first 6 years of life had in fact IgE levels higher than 0.9 kU/L at birth. The same was true for about half of the children showing atopic symptoms at some occasion during the same period.

Prediction of allergy is of course meaningful only if preventive measures can be recommended to the parents (142). Such recommendations have been developed by health care authorities in some countries. These include prolonged breast feeding and elimination of inhalation allergens and tobacco smoke. Smoking during pregnancy should also be avoided since it increases the risk of allergy even in nonatopic women (143).

Duchateau and Casimir (144) reported encouraging results in breast-fed babies as compared with babies who received cow's milk during the first month of life. A very high proportion of the children with elevated IgE levels at birth developed symptoms if fed with cow's milk. A similar but breast-fed group was essentially free of symptoms. Control groups with low IgE, i.e., less than 1 kU/L, seemed not to be affected by feeding. The authors recommended general screening of all newborn babies justified by the around 10% frequency of elevated IgE in the total newborn population, the importance of a high compliance to the preventive measures, and the trauma and cost caused to the patient and society if the disease is permitted to develop at an early age. Similar data were presented by Businco et al. (145) and Chandra et al. (146).

Serum Immunoglobulin E Levels in Nonallergic Diseases

In addition to atopic diseases, high IgE concentrations are regularly seen in certain parasitic infestations caused by helminths such as ascaris, toxocara, schistosoma, hook worm, and others (147). The IgE is the result of a combination of a specific IgE antibody production and a nonspecific adjuvant effect. The IgE levels often are higher than in atopic allergy, in rare cases more than 100,000 kU/L, but decrease significantly within months of an effective treatment (148).

Increased serum IgE concentrations have also been found in certain viral infections such as Epstein-Barr virus (149), cytomegalovirus (150) and mononucleosis. Here, a time-dependent variation with the highest values after a couple of weeks of infection is seen although many cases never exceed the normal IgE range. It is likely that the viral infection interferes with mechanisms for IgE production control in a nonantigen-specific way.

In the graft vs host reaction, often after a bone marrow transplantation, an IgE peak covering 5–10 d is often seen (151). The IgE increase from baseline can be considerable, sometimes several hundredfold. No antigen specificity has been found and the IgE production is most likely a result of disturbances in production control. A similar phenomenon has been reported in rare cases of combined immunodeficiency and in the late stages of certain malignant diseases (147).

Specific Immunoglobulin E Antibody in Relation to Disease State and In Vivo Tests

Allergy is a complex of conditions presenting itself in a variety of ways. The different types of disorders often occur simultaneously in the allergic patient, presenting difficult differential diagnostic problems that have to be solved before the disease can be adequately treated. Some major events in the development of an allergic response (152) are summarized in Fig. 3.

Atopic allergy is traditionally diagnosed by introducing extracts of possible allergen sources into the skin, the nose, the eyes, or the bronchi, i.e., by skin or provocation tests. It is well known that the

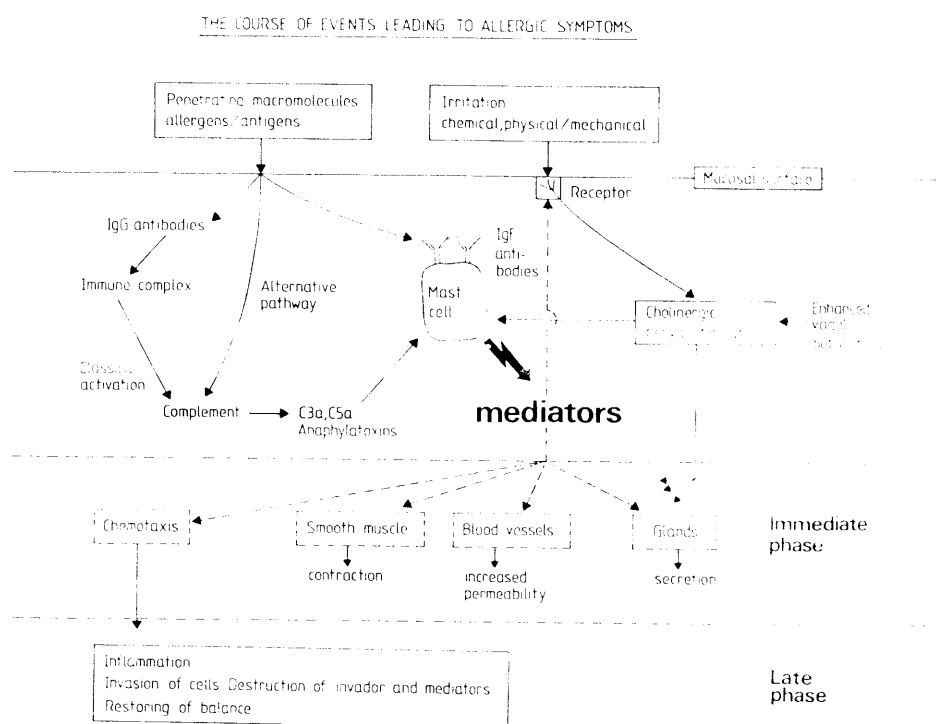


Fig. 3. Some mechanisms potentially involved in immediate and late-phase reactions to external stimuli.

reliability of skin and provocation tests is highly dependent on the quality and potency of the allergen preparations used. Numerous authors have commented on the fact that high concentrations of allergen, particularly upon intradermal administration, frequently also provoke reactions in individuals free of allergic symptoms. It has been shown that such reactions are most frequent with allergens originating from epithelia, fungi, and foods. The difficulties with the skin test are clearly illustrated in the studies by Toogood (153) showing that an allergen concentration high enough to detect all sensitive individuals (100% sensitivity) will also give a high proportion of positive reactions in nonsensitive individuals. In the case shown in Fig. 4, about 60% false-positive reactions were obtained, i.e., a very poor specificity. One explanation for this situ-

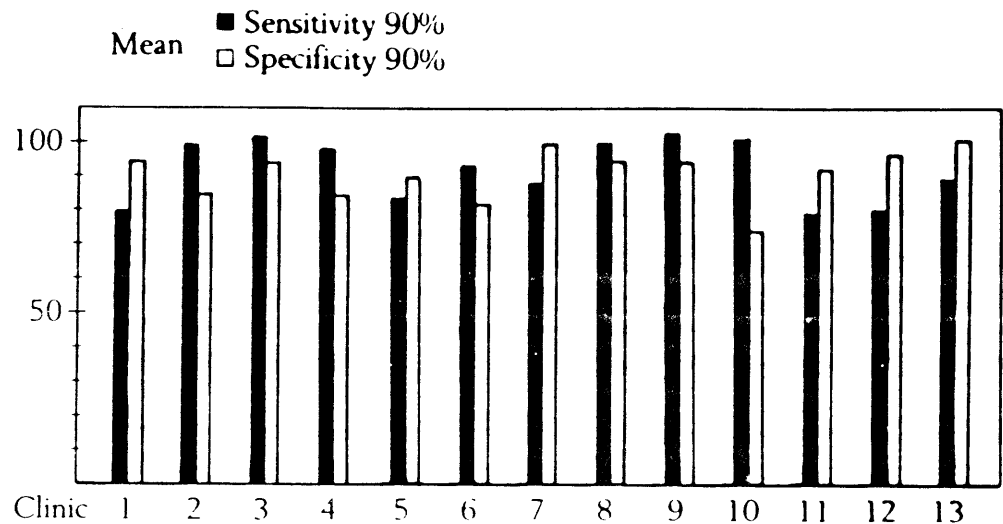


Fig. 5. Sensitivity and specificity of Phadiatop. A summary of results from 13 clinical trials (286).

hour laboratory test (radioimmunoassay or enzyme immunoassay) that applies a combination of inhalant allergens selected on the basis of knowledge and experiences about the situation in different geographical areas regarding: local aerobiology, potential exposure, immunochemical characteristics, immunologic cross-reactions, sensitizing capacity, and clinical relevance.

The results of the first 13 of a series of clinical trials comparing Phadiatop with the judgment of the clinician based on his routine in terms of tests and diagnostic criteria show an average sensitivity as well as specificity of 90% in groups of patients with respiratory symptoms (277) (Fig. 5). The variation between the trials reflects the variation between the physicians' judgments and is not a variation in the test performance. It is hoped that a test like this will contribute to decreasing the number of individuals suffering from an undiagnosed allergy by providing a decision aid for the differential diagnosis at the primary-care level.

REFERENCES

1. Johansson, S. G. O., Bennich, H., and Wide, L. (1968), *Immunology* **14**, 265-272.
2. Wide, L., Bennich, H., and Johansson, S. G. O. (1967), *Lancet* **ii**, 1105.
3. Wide, L. and Porath, J. (1966), *Biochim. Biophys. Acta.* **130**, 257-260.
4. Gleich, G. J. and Dunnette, S. L. (1977), *J. Allergy Clin. Immunol.* **59**, 377-382.
5. Kober, A. and Uppström, L. (1985), *Allergy* **40** (Suppl. 4), 12-14.
6. Guilloux, L., Hartmann, F. J., and Ville, G. (1986), *EAACI Abstracts*, Budapest, p. 31.
7. Ceska, M. and Lundkvist, U. (1972), *Immunochemistry* **9**, 1021-1030.
8. Hemady, Z., Blomberg, F., Gillis, S., and Rocklin, R. E. (1983), *J. Allergy Clin. Immunol.* **71**, 324-330.
9. Schröder, H., Kober, A., and Yman, L. (1981), *Allergol. Immunopathol. Suppl.* **9**, 46-50.
10. Magnusson, S. G. M., Collet-Cassart, D., Merrett, T. G., and Masson, P. L. (1981), *Clin. Allergy* **11**, 453-461.
11. Phillips, T. M., More, N. S., Queen, W. D., and Thompson, A. M. (1985), *J. Chromatogr.* **327**, 205-211.
12. Foucard, T., Bennich, H., Johansson, S. G. O., and Lundkvist, U. (1975), *Int. Arch. Allergy Appl. Immunol.* **48**, 812-823.
13. Zaunders, J. J., Ziegler, J. B., Penny, R., and Cooper, D. A. (1985), *Int. Arch. Allergy Appl. Immunol.* **78**, 1-8.
14. Spiegelberg, H. L., Plummer, J. M., Ruedi, J., Thompson, L. F., Mellon, M. H., and Zeiger, R. S. (1983), *J. Immunol.* **131**, 3001-3005.
15. Deuschl, H. and Johansson, S. G. O. (1977), *Clin. Allergy* **7**, 195-202.
16. Grassi, J., Didierlaurent, A., and Stadler, B. M. (1986), *J. Allergy Clin. Immunol.* **77**, 808-822.
17. Conway, De Macario E., Macorio, A. J. L., and Jowell, R. J. (1986), *J. Immunol. Methods* **90**, 137-141.
18. Poulsen, K. L., Malling, H. J., Sondergaard, I., and Weeke, B. N. (1986), *J. Immunol. Methods* **92**, 131-136.
19. Struy, H., Schuster, R., Sollich, V., Thal, W., and Morenz, J. (1984), *Dtsch. Gesundheits-wes.* **37**, 1461-1464.
20. Subba Rao, P. V., McCartney-Francis, N. L., and Metcalfe, D. D. (1983), *J. Immunol. Methods* **57**, 71-85.
21. Reid, M. J., Kwasnicki, J. M., Moss, R. B., and Cheung, N. K. V. (1985), *J. Allergy Clin. Immunol.* **76**, 172-176.
22. Houben, L. A. M. J. and Bruynzeel, P. L. B. (1982), *J. Clin. Chem. Clin. Biochem.* **20**, 553-556.
23. Wüthrich, B. and Marti-Wyss, S. (1982), *Allergol. Immunopathol.* **10**, 463-470.
24. Bienvenu, J., Bienvenu, F., Michand-Candie, C., Quincy, C., Nicolas, J. P., Phang, P. P., Mandrand, B., and Trabuc-Jaubert, M. (1982), *Ann. Clin. Biochem.* **19**, 60-63.
25. Diel, F. (1981), *Lab. Med.* **5**, 67-72.

26. Derer, M. M., Miescher, S., Johansson, B., Frost, H., and Gordon, J. (1984), *J. Allergy Clin. Immunol.* **74**, 85-92.
27. Guesdon, J. L., David, B., and Lapeyre, J. (1978), *Clin. Exp. Immunol.* **33**, 430-436.
28. Bourgeois, J. P., Pére, M., Mazié, J. C., and Delagneau, J. F. (1984), *Dev. Biol. Stand.* **57**, 371-379.
29. Hartmann, D. J., Bourgeois, J. P., Mazie, J. C., Ville, G., Delagneau, J. F., Guilloux, L., and Paternostre, B. (1985), *Eur. J. Nucl. Med.* **11**, A22.
30. Cukor, P., Woehler, M. E., Persiani, C., and Fermin, A. (1976), *J. Immunol. Methods* **12**, 183-192.
31. Blanchard, G. C. and Gardner, R. E. (1982), *J. Immunol. Methods* **52**, 81-90.
32. Lee, S.-Y., Chen, J.-S., Cook, W. E., and Tung, K.-K. (1983), *Clin. Biochem.* **16**, A13-14.
33. Thorpe, G. H. G., Williams, L. A., Kricka, L. J., Whitehead, T. P., Evans, H., and Stanworth, D. R. (1983), *J. Immunol. Methods* **79**, 57-63.
34. Underdown, B. J., James, O., and Knight, A. (1983), *J. Allergy Clin. Immunol.* **71**, 122.
35. Cingolani, M., Penna, R., di Stefano, A., Fortini, P., Nattero, G., Botticelli, A. M., Merlini, M., and Risso, D. (1983), *Minerva Pediatr.* **35**, 27-34.
36. Belaube, B., Roux, F., Belaube, P., and Bisset, J. P. (1982), *Pathol. Biol.* **30**, 217-219.
37. Pecoud, A., Peitrequin, R., Duc, J., Thalberg, K., Schröder, H., and Frei, P. C. (1986), *Clin. Allergy* **16**, 231-239.
38. Schröder, H., Edman, P., and Johansson, S. G. O. (1986), *Allergy* **41**, 163-165.
39. Brown, C. R., Higgins, K. W., Grazer, K., Schoelz, L. K., Dyminski, J. W., Marinkovich, V. A., Miller, S. P., and Burd, J. F. (1985), *Clin. Chem.* **31**, 1500-1505.
40. Bazaral, M. and Hamburger, R. N. (1972), *J. Allergy Clin. Immunol.* **49**, 189-191.
41. Johansson, S. G. O., Berglund, A., and Kjellman, N.-I. M. (1976), *Clin. Allergy* **6**, 91-98.
42. Ryal, R. G., Story, C. J., and Turner, D. R. (1982), *Anal. Biochem.* **127**, 308-315.
43. Williams, Jr., R. C., Griffiths, R. W., Emmons, J. D., and Field, R. C. (1972), *Clin. Invest.* **51**, 955.
44. Inganäs, M., Johansson, S. G. O., and Bennich, H. (1981), *Int. Arch. Allergy Appl. Immunol.* **65**, 51.
45. Merety, K., Falus, A., Erhardt, C. C., and Maini, R. N. (1982), *Ann. Rheum. Dis.* **41**, 405.
46. Camussi, G., Tetta, C., and Benveniste, J. (1982), *Int. Arch. Allergy Appl. Immunol.* **69**, 358-362.
47. Ceska, M., Eriksson, R., and Varga, J. M. (1972), *J. Allergy* **49**, 1-9.

48. De Filippi, I., Yman, L., and Schröder, H. (1981), *Ann. Allergy* **46**, 249–255.
49. Pecoud, A., Ochsner, M., Arrendal, H., and Frei, P. C. (1982), *Clin. Allergy* **12**, 75–81.
50. Vooren, P. H., Kramps, J. A., Franken, C., and Dijkman, J. H. (1983), *Eur. J. Respir. Dis.* **64**, 90–101.
51. Dominquez, J., Cuevas, M., Moneo, I., Urêna, V., Ferreira, A., and Bootello, A. (1984), *Allergy* **39**, 529–533.
52. Beaulieu, L., Valet, J. P., Beaudoin, J., Boulet, A., Jobin, M., Perelmutter, L., and Hébert, J. (1986), *J. Immunol. Methods* **86**, 131–138.
53. Aalberse, R. C., Van Zoonen, M., Clemens, J. G. J., and Winkel, I. (1986), *J. Immunol. Methods* **87**, 51–57.
54. Platts-Mills, T. A. E., Snajdr, M. J., Ishizaka, K., and Frankland, A. W. (1978), *J. Immunol.* **120**, 1201–1210.
55. Svejgaard, E. and Loewenstein, H. (1986), *Acta. Derm. Venereol.* (Suppl) **120**, 72–75.
56. Hoffman, D. (1983), *J. Allergy Clin. Immunol.* **71**, 122.
57. Siraganian, R. P. and Hook, W. A. (1976), *J. Immunol.* **116**, 639–646.
58. Benveniste, J. (1977), The In Vitro Basophil Degranulation Test, *Excerpta Medica*, Mathov, E., Sindo, T., and Naranjo, P. (eds.), Amsterdam, pp. 418–419.
59. Sánchez-Madrid, F., Morago, G., Corbi, A. L., and Carreira, J. (1984), *J. Immunol. Methods* **73**, 367–378.
60. Bergland, A., Kober, A., Ahlberg, M., and Persson, E. (1985), *Allergy* **40**, (Suppl 4), 18.
61. Ali, M. and Ramanarayanan, M. P. (1984), *Ann. Allergy* **53**, 144–150.
62. Plebani, A., Notarangelo, L. D., Monafo, V., Nespoli, L., and Ugazi, A. G. (1984), *Clin. Allergy* **14**, 373–378.
63. Greenberger, P. A. and Patterson, R. (1982), *J. Lab. Clin. Med.* **99**, 288–293.
64. Metzger, W. J., Butler, J. E., Swanson, P., Reinders, E., and Richerson, H. B. (1981), *Clin. Allergy* **11**, 523–531.
65. Seltzer, J. M., Halpern, G. M., and Tsay, Y.-G. (1985), *Ann. Allergy* **54**, 26–30.
66. Bergeron, M. and Perelmutter, L. (1983), *J. Allergy Clin. Immunol.* **71**, 122.
67. Jacob, G. L., Ownby, D. R., and Homburger, H. A. (1983), *J. Allergy Clin. Immunol.* **71**, 122.
68. Leonhardt, L. and Deycke, K. L. (1986), *EAACI Abstracts*, Budapest, p. 15.
69. Kersten, W. and Burow, G. (1987), *EAACI Abstracts*, Budapest, p. 16.
70. Henseler, T. (1987), *Allergologie* **10**, 124–127.
71. Viander, M., Koivikko, A., Sved, D., and Einarsson, R. (1986), *EAACI Abstracts*, Budapest, p. 19.
72. Merrett, J. and Merrett, T. G. (1978), *Clin. Allergy* **8**, 235–240.
73. Gleich, G. J. and Jones, R. T. (1975), *J. Allergy Clin. Immunol.* **55**, 334–345.

74. Aalberse, R. C. (1974), *Ph.D. Thesis*, Amsterdam.
75. Poulsen, L. K. and Weeke, B. (1985), *Allergy* **40**, 405-416.
76. Grunewalder, E. and Karol, M. H. (1986), *Allergy* **41**, 203-209.
77. Walsh, B. J., Wrigley, C. W., and Baldo, B. A. (1984), *J. Immunol. Methods* **66**, 99-102.
78. Blessing-Moore, J., Ried, M., Hsu, Y., Leahy, M., Babin, S., Kwasnicki, M., and Cheung, N. (1982), *Ann. Allergy* **48**, 266.
79. Pécoud, A., Peitrequin, R., Fasel, J., and Frei, P. C. (1986), *Allergy* **41**, 243-249.
80. Plebani, A., Avanzini, M. A., Scotta, M. S., Monafò, V., Guiunta, A. M., Guandalini, S., Ugazio, A. G., and Burgio, R. G. (1986), *J. Clin. Lab. Immunol.* **20**, 93-96.
81. Perelmutter, L. and Emanuel, I. (1985), *Ann. Allergy* **55**, 762-766.
82. Schröder, H., Thalberg, K., and Lundberg, A. (1985), *Allergy* **40** (Suppl. 4), 14-17.
83. Yman, L., Blomberg, F., and Schröder, H. (1980), *Advances in Allergology and Immunology*, Oehling, A. (ed.), Pergamon Press, Oxford, pp. 499-504.
84. Peltre, G., Lapeyre, J., and David, B. (1982), *Immunol. Lett.* **5**, 127-131.
85. Hamilton, R. G. and Adkinson, N. F. (1986), *Clin. Lab. Med.* **6**, 117-138.
86. Lundkvist, U. (1975), *Advances in Diagnosis of Allergy: RAST*, Evans III, R. (ed.), Symposia Specialists, Miami, pp. 85-99.
87. Evans, R. (1982), *J. Allergy Clin. Immunol.* **69**, 245-252.
88. Schröder, H. and Thalberg, K. (1985), *Allergy* **40** (Suppl. 4), 19, 20.
89. Westgard, J. O., Barry, P. L., Hunt, M. R., and Groth, T. (1981), *Clin. Chem.* **27**, 493-501.
90. Johansson, S. G. O. and Deuschl, H. (1976), *Int. Arch. Allergy Appl. Immunol.* **52**, 364-375.
91. Ohashi, Y., Nakai, Y., Kuroki, K., Nakata, J., Maruoka, K., Ikeoka, H., Takeichi, N., and Kihara, S. (1985), *Arch. Otorhinolaryngol.* **241**, 169-174.
92. Small, P., Barrett, D., Frenkiel, S., Rochon, L., Cohen, C., and Black, M. (1985), *Ann. Allergy* **55**, 68-71.
93. Nickelsen, J. A., Georgitis, J. W., and Reisman, R. E. (1986), *J. Allergy Clin. Immunol.* **77**, 43-48.
94. Liotet, S., Warnet, V. N., and Arrata, M. (1983), *Ophthalmologica* **186**, 31-34.
95. Sompolsky, D., Samra, Z., Zavaro, A., and Barishak, Y. (1984), *Int. Arch. Allergy Appl. Immunol.* **75**, 317-321.
96. Kari, O., Salo, O. P., Björkstén, F., and Backman, A. (1985), *Acta. Ophthalmol.* **63**, 97-99.
97. Bloch-Michel, E., Liotet, L., Lecorvec, M. P., and Serquetti, P. M. (1985), *Rev. Fr. Allergol.* **25**, 135-139.
98. Aalders-Deenstra, V., Kok, P. T. M., and Bruynzeel, P. L. B. (1985), *Br. J. Ophthalmol.* **69**, 380-384.
99. Ballow, M. and Mendelson, L. (1980), *J. Allergy Clin. Immunol.* **66**, 112-118.

100. Marcucci, F., Sensi, L. G., and Bizzarri, G. (1985), *Clin. Allergy* **15**, 345-354.
101. Donovan, R., Johansson, S. G. O., Bennich, H., and Soothill, J. F. (1970), *Int. Arch. Allergy* **37**, 154.
102. Small, P., Barrett, D., Frenkiel, S., Rochon, L., Cohen, C., and Black, M. (1985), *Ann. Allergy* **55**, 736-739.
103. Ohashi, Y., Nakai, Y., Nakata, J., Kihara, S., and Ikeoka, H. (1986), *ORL J. Otorhinolaryngol. Relat. Spec.* **48**, 233-237.
104. Crimi, E., Scordamaglia, A., Crimi, P., Zupo, S., and Barocci, S. (1983), *Allergy* **38**, 553-559.
105. Nash, D. R. and Wallace, R. J. (1985), *J. Lab. Clin. Med.* **106**, 512-516.
106. Bernstein, J. M., Lee, J., Conboy, K., Ellis, E., and Li, P. (1985), *Otolaryngol. Head Neck Surg.* **93**, 611-615.
107. Sindic, C. J., Magnusson, C. G., Laterre, E. C., and Masson, P. L. (1984), *J. Neuroimmunol.* **6**, 319-324.
108. Kolmannskog, S., Marhaug, G., and Haneberg, B. (1985), *Int. Arch. Allergy Appl. Immunol.* **78**, 358-363.
109. Magnusson, C. G. M. and Masson, P. L. (1985), *Int. Arch. Allergy Appl. Immunol.* **77**, 292-299.
110. Zimmerman, E. M., Yunginger, J. W., and Gleich, G. J. (1980), *J. Allergy Clin. Immunol.* **66**, 386-393.
111. Fujita, Y., Wicher, K., Wypych, J. I., Reisman, R. E., and Arbesman, C. E. (1975), *Int. Arch. Allergy Appl. Immunol.* **49**, 636-646.
112. Nelson, H. S. (1980), *Ann. Allergy* **45**, 333-337.
113. Zeiss, S. R., Grammer, L. C., and Levitz, D. (1981), *J. Allergy Clin. Immunol.* **67**, 105-110.
114. Perelmutter, L., Bergeron, M., and Mandy, F. (1983), *Ann. Allergy* **50**, 393-397.
115. Hamilton, R. G. and Adkinson, N. F. (1982), *Immunopathology* **6**, 1-8.
116. Squire, E. N., McBride, D. C., Ledoux, R., and Nelson, H. S. (1986), *Ann. Allergy* **56**, 22-27.
117. Nye, L., Merrett, T. G., Landon, L., and White, R. J. (1975), *Clin. Allergy* **5**, 13-24.
118. Sears, M. R., Chow, C. M., and Morseth, D. J. (1980), *Clin. Allergy* **10**, 423-431.
119. Barbee, R. A., Halonen, M., Lebowitz, M., and Burrows, B. (1981), *J. Allergy Clin. Immunol.* **68**, 106-111.
120. Zetterström, O., Osterman, K., Machado, L., and Johansson, S. G. O. (1981), *Br. Med. J.* **283**, 1215-1217.
121. Warren, P. P. W., Holford-Strevens, V., Wong, C., and Manfreda, J. (1982), *J. Allergy Clin. Immunol.* **69**, 370-375.
122. Haahtela, T., Suoniemi, I., Jaakonmäki, I., and Björkstén, F. (1982), *Allergy* **37**, 597-602.
123. Furukawa, T. (1982), *Pediatr. Jp.* **23**, 555.
124. Burrows, B., Halonen, M., Barbee, R. A., and Lebowitz, M. D. (1981), *Am. Rev. Respir. Dis.* **124**, 523-525.

125. Merrett, T. G., Merrett, J., and Cookson, J. B. (1976), *Clin. Allergy* **6**, 131-134.
126. Ganju, A., Baram, P., and Lim, D. T. (1979), *Ann. Allergy* **43**, 271-274.
127. Kjellman, N.-I. M., Johansson, S. G. O., and Roth, A. (1976), *Clin. Allergy* **6**, 51-59.
128. Bruynzeel, P. L. B. and Houben, L. A. M. J. (1981), *Clin. Chem. Acta.* **112**, 315-323.
129. Witting, H. J., Belloit, J., De Filippi, I., and Royal, G. (1980), *J. Allergy Clin. Immunol.* **66**, 305-313.
130. Bhalla, R. B., Rappaport, I., De Filippi, I., and Schwartz, M. K. (1982), *Advanced Interpretation of Clinical Laboratory Data*, (Heusghem, C., Albert, A., and Benson, E. S., eds.), Marcel Dekker, New York, New York, pp. 295-305.
131. Zetterström, O. and Johansson, S. G. O. (1981), *Allergy* **36**, 537-547.
132. Berg, T. and Johansson, S. G. O. (1971), *Int. Arch. Allergy* **41**, 452-462.
133. Berg, T. and Johansson, S. G. O. (1971), *Int. Arch. Allergy* **41**, 434-442.
134. Björkstén, F. and Johansson, S. G. O. (1975), *Clin. Allergy* **5**, 363-373.
135. Hamburger, R. N. (1974), *Allergol. Immunopathol.* **2**, 101-106.
136. Kjellman, N.-I. M. (1976), *Acta. Paediatr. Scand.* **65**, 465-471.
137. Businco, L., Fredani, T., Lucarelli, S., Finocchi, M., Puddu, M., and Businco, E. (1979), *Ann. Allergy* **43**, 120-122.
138. Michel, F. B., Bousquet, J., Greillier, P., Robinet-Levy, M., and Coulomb, Y. (1980), *J. Allergy Clin. Immunol.* **65**, 422-430.
139. Croner, S., Kjellman, N.-I. M., Ericksson, B., and Roth, A. (1982), *Arch. Dis. Childhood* **57**, 364-368.
140. Bousquet, J., Ménardo, J.-L., Viala, J.-L., and Michel, F. B. (1983), *Ann. Allergy* **51**, 291-295.
141. Kjellman, N.-I. M. and Croner, S. (1984), *Ann. Allergy* **53**, 167-171.
142. Björkstén, B. (1983), *Immunol. Today* **4**, 215-217.
143. Magnusson, C. G. M. (1986), *J. Allergy Clin. Immunol.* **78**, 898-904.
144. Duchateau, J. and Casimir, G. (1983), *Lancet* **i**, 413, 414.
145. Businco, L., Marchetti, F., Pellegrini, G., Cantani, A., and Perlini, R. (1983), *Ann. Allergy* **51**, 296-299.
146. Chandra, R. K., Puri, S., and Cheema, P. S. (1985), *Clin. Allergy* **15**, 517-522.
147. Johansson, S. G. O. (1981), *Clinical Immunology Update*, (Franklin, E. C., ed.), Elsevier, New York, pp. 123-145.
148. Rosenberg, E. B., Whalen, G. E., Bennich, H., and Johansson, S. G. O. (1970), *N. Engl. J. Med.* **283**, 1148, 1149.
149. Nordbring, F., Johansson, S. G. O., and Espmark, Å. (1972), *Scand. J. Infect. Dis.* **4**, 119-124.
150. Bahna, S. L., Horwitz, C., and Heiner, D. C. (1978), *J. Allergy Clin. Immunol.* **61**, 177.
151. Ringden, O., Persson, U., and Johansson, S. G. O. (1983), *Immunol. Rev* **71**, 57.
152. Austen, K. F. (1979), *Harvey Lect.* **73**, 93.
153. Toogood, J. H. (1983), *Ann. R. Coll. Physicians Surg. (Canada)* **16**, 310.

154. Stevens, W. J., van Schil, L., and Vermeire, P. A. (1983), *Allergol. Immunopathol.* **11**, 243-249.
155. Spieksma, F. T. M. (1983), *Grana* **22**, 119-128.
156. Spieksma, F. T. M., van den Assem, A., and Collette, B. J. A. (1985), *Grana* **24**, 99-108.
157. Spieksma, F. T. M. (1986), *Grana* **25**, 47-54.
158. Diener, C., Schlenvoigt, G., Jager, L., Prater, E., and Schubert, H. (1986), *Allergol. Immunopathol.* **14**, 49-53.
159. Axelsson, J. G. K., Johansson, S. G. O., and Zetterström, O. (1987), *Allergy* **42**, 604-611.
160. Halse, R. R. (1984), *Ann. Allergy* **53**, 291-309.
161. Weber, R. W. and Nelson, H. S. (1985), *Clin. Rev. Allergy* **3**, 291-318.
162. Yman, L. (1982), *Botanical Relations and Immunological Reactions in Pollen Allergy*, Pharmacia Diagnostics AB, Uppsala, Sweden.
163. Walker, I. C. (1971), *J. Med. Res.* **35**, 497-503.
164. Anderson, M. C. and Baer, H. (1981), *J. Immunol.* **127**, 972-975.
165. Ohman Jr., J. L. (1982), *J. Allergy Clin. Immunol.* **69**, 127.
166. Hoffman, D. R. (1980), *Ann. Allergy* **45**, 205, 206.
167. Lutsky, I., Fink, J. A., Hoffman, D. R., and Morouse, M. (1980), *Clin. Allergy* **10**, 331-340.
168. Ohman Jr., J. L. (1978), *J. Am. Vet. Med. Assoc.* **172**, 1403-1406.
169. Brandt, R. and Yman, L. (1980), *Int. Arch. Allergy Appl. Immunol.* **61**, 361-370.
170. Vanto, T., Viander, M., Koivikko, A., Schwartz, B., and Loewenstein, H. (1982), *Allergy* **37**, 75-85.
171. Leegaard, J. and Roth, A. (1977), *Clin. Allergy* **7**, 455-464.
172. Yman, L., Brandt, R., and Ponterius, G. (1973), *Int. Arch. Allergy Appl. Immunol.* **44**, 358-368.
173. Brandt, R., Ponterius, G., and Yman, L. (1973), *Int. Arch. Allergy Appl. Immunol.* **45**, 447-455.
174. Ponterius, G., Brandt, R., Hultén, E., and Yman, L. (1973), *Int. Arch. Allergy Appl. Immunol.* **44**, 679-691.
175. Ohman Jr., J. L. (1973), *J. Allergy Clin. Immunol.* **52**, 231-241.
176. Ohman Jr., J. L., Bloch, K. J., and Kendall, S. (1976), *J. Allergy Clin. Immunol.* **57**, 560-568.
177. Hooker, S. B. (1944), *Ann. Allergy* **2**, 281-288.
178. Faberberg, E. and Wide, L. (1970), *Int. Arch. Allergy* **39**, 301-309.
179. Moore, B. S., Hyde, J. S., and Manaligod, L. M. (1977), *Ann. Allergy* **39**, 240-245.
180. Blands, J., Loewenstein, H., and Weeke, B. (1977), *Acta. Allergol.* **32**, 147-169.
181. Wüthrich, B., Guerin, B., and Hewitt, B. (1985), *Clin. Allergy* **15**, 87-93.
182. Uhlin, T., Reuterby, J., and Einarsson, R. (1984), *Allergy* **39**, 125-133.
183. Viander, M., Valovirta, E., Vanto, T., and Koivikko, A. (1983), *Int. Arch. Allergy Appl. Immunol.* **71**, 252-260.
184. Björkstén, F. and Johansson, S. G. O. (1975), *Clin. Allergy* **5**, 363-373.
185. Vanto, T. and Koivikko, A. (1983), *Acta. Paediatr. Scand.* **72**, 571-575.