

Serum IgE and other *in vitro* tests in drug allergy

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Summary

A study has been carried out in order to establish the diagnostic value of serum IgE estimation and other *in vitro* tests in drug allergy. The comparative value of these tests in allergic asthma and rhinitis has also been assessed. Since there were fewer problems in the diagnosis of the latter conditions, serum IgE estimation was of relatively greater value in the diagnosis of drug allergy, although raised IgE was found only in twenty-three of sixty-two (37%) patients. The overall incidence of raised IgE in allergic asthmatics (20%) was lower than in previous reports, and like in drug allergy, the frequency was smaller in patients with a single allergy. In drug allergy, the importance of using proper material in the various tests was illustrated in penicillin allergy. Skin tests and *in vitro* correlates of immediate-type allergy frequently gave false negative results with the drug itself rather than its conjugated derivatives (penicilloyl-polylysine and penicilloyl-human serum albumin), while in the lymphocyte stimulation (transformation) test this limitation was not as marked. The lymphocyte stimulation test was positive in the majority of patients, both in drug allergy and in allergic asthma and rhinitis, and on the whole did not seem to correlate with the type of reaction (whether immediate or delayed) to *in vivo* testing.

Introduction

Allergic drug reactions occur frequently and it is often difficult to establish the diagnosis. Several *in vitro* tests have been used, but no single test has proved entirely reliable, mainly because of the lack of proper antigens (Assem, 1967).

Immediate-type allergy, which is mediated by reaginic antibodies (mainly IgE) may be detected by the following tests: (1) *in vitro* passive sensitization of various tissues, e.g. human lung (Assem & Schild, 1968); (2) *in vitro* challenge of isolated leucocytes of patients (Lichtenstein & Osler, 1964); (3) the radio-allergosorbent test (RAST; Wide, Bennich & Johansson, 1967). In the RAST analysis the allergen is conjugated to an insoluble carrier and this conjugate is used for the detection of allergen-specific IgE.

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In allergy to drugs which are simple chemicals (molecular weight less than 1000) the RAST analysis is at present of limited value. One of the main reasons for this is that the drug itself, or its various metabolites which seem to be more important in the production of allergy, act only as haptens. The preparation of hapten-carrier conjugates in an insoluble form, which is essential for the RAST procedure, is a big problem. To our knowledge the RAST analysis has so far been applied only in penicillin allergy by Wide & Juhlin (1970), who used this technique to detect serum IgE antibodies against the penicilloyl group (the main haptenic determinant formed during the degradation of penicillin), using phenoxymethyl-penicilloyl polylysine conjugated to an insoluble polysaccharide (Sephadex or cellulose) as an immunosorbent.

Over the past 5 years we have investigated many patients with single or multiple drug allergies, using a variety of *in vitro* tests, described below. One of the main purposes of this paper has been to assess the value of serum IgE estimation in patients with established drug allergy using the radioimmunosorbent test (RIST). This test, unlike the RAST, detects total IgE rather than allergen-specific IgE. Though non-specific, the RIST is simpler than the RAST because it does not require the preparation of suitable conjugates from haptens. The present work differs in several respects from previous reports on serum IgE in patients with drug allergy (e.g. Juhlin *et al.*, 1969) by including larger numbers of patients, in the majority of whom the diagnosis of allergy has been supported by a variety of *in vitro* tests.

Material and methods

Patients with drug allergy

These patients were divided into various groups according to the drugs involved, whether one or more drugs were implicated, and whether the patient had an associated atopic condition (Table 1). Patients with penicillin allergy comprised the main group.

Patients with respiratory allergy

Fifty patients with established allergy to one or more inhalant allergens (mainly grass pollen and the house-dust mite, *D. pteronyssinus*) were included for comparative purposes. Serum from the pollen-allergic patients was collected in January. Immediate-type allergy in all patients was established by five *in vivo* and *in vitro* test parameters (McAllen, Assem & Maunsell, 1970).

Non-allergic controls

Sixty-three subjects with no personal or family history of allergy were used as controls. Serum IgE was determined in all, but only eleven of these have been investigated by skin and *in vitro* testing for allergy to penicillin, grass pollen and *D. pteronyssinus*.

Skin tests

Tests for penicillin allergy were carried out by intradermal injections of benzylpenicillin and, when required, other penicillin derivatives. Penicilloyl conjugates with polylysine and human serum albumin were also used in skin tests. In allergy to drugs other than penicillin intradermal tests with solutions of the drug itself (not conjugates) were carried out.

Detection of serum reagins

This was carried out by passive sensitization and histamine release from chopped human lung tissue (Assem & Schild, 1968). Lung tissue was obtained from surgical operations on patients with lung cancer. A positive result means a histamine release from sensitized tissue of at least one and a half times that from non-sensitized aliquots.

Allergen-induced histamine release from leucocytes

Leucocytes isolated from heparinized venous blood samples were challenged with allergen and the histamine released in this reaction was measured by bioassay on guinea-pig ileum as described by Assem & Schild (1968). The experimental procedure is a modification of the technique originally described by Lichtenstein & Osler (1964). Positive results were indicated by the release from allergen challenged leucocytes of at least twice as much histamine as from unchallenged cell aliquots.

In some patients and control subjects isolated leucocytes were challenged with anti-IgE serum in the so-called reversed anaphylaxis test (Assem & McAllen, 1970) and histamine released in this way was estimated. This test detects cell-fixed IgE.

Lymphocyte stimulation (transformation) test

Both the detection of reagins by passive sensitization of human lung and leucocyte challenge tests previously mentioned are *in vitro* correlates of immediate-type hypersensitivity. The lymphocyte transformation test is considered to be an *in vitro* correlate of delayed-type hypersensitivity. Since, however, a positive lymphocyte transformation test may be obtained in conditions which are considered as classical examples of immediate-type allergy (Girard *et al.*, 1967; Fellner *et al.*, 1967) the results of the lymphocyte stimulation test in some of the patients have also been included. This test was carried out according to the method described by Chalmers *et al.* (1967). In short, venous blood samples were defibrinated and then decanted into bottles containing carbonyl iron particles. Methyl cellulose (1% solution in 0.9% NaCl) was added to the defibrinated blood in a ratio of 1:3. The red cells and the leucocytes engulfing the carbonyl iron particles were separated by sedimentation, and the lymphocyte-rich supernatant was transferred to tissue culture tubes. Tissue culture medium 199 (Wellcome) containing 10% autologous serum was used and the lymphocytes were incubated with various concentrations of antigen for 4 days, following which the incorporation of ^3H -thymidine, over the period of 1 hr, was measured. The test is considered positive when incorporation of ^3H -thymidine is significantly higher in allergen-stimulated aliquots than in aliquots not treated with allergen.

Antigens

In penicillin allergy *in vitro* tests were carried out with at least two test materials, benzylpenicillin and benzylpenicilloyl polylysine. In allergy to other drugs, the tests were carried out with the unmodified drug.

Estimation of serum IgE

This was carried out by the radioimmunosorbent technique (RIST), using anti-IgE antibodies conjugated to cyanogen bromide-activated Sephadex particles and ^{125}I -labelled human IgE. Kits for this test were generously donated by Pharmacia Ltd. The manufacturers recommend the expression of IgE values in units. However, since

one unit is said to be roughly equivalent to one nanogram we decided to express serum IgE in nanograms/ml.

Results

Serum IgE in drug allergy

In the sixty-three non-allergic controls the arithmetic mean of serum IgE was 225 ng/ml, the range varied widely between 1 and 1700 ng/ml, and the standard deviation was 320. Only two of these controls had an IgE higher than 900 ng/ml (an approximation of the mean value plus two standard deviations) (Fig. 1). In allergic patients IgE levels varied within a much wider range (1–29,000 ng/ml) rendering arithmetic means and standard deviations meaningless. Thus, a logarithmic transformation to base 10 was used in preference to the arithmetic values (Table 1). Statistical analyses of transformed values were carried out, using Student's *t* test.

Serum IgE was higher than 900 ng/ml in nine of the thirty-eight patients (24%) with penicillin allergy only, in three of seven patients with allergy to penicillin and other drugs, and in all of the four patients with allergy to penicillin as well as to common inhalent allergens (Table 1).

Serum IgE was also raised in four of the six patients with allergy to anti-tuberculous drugs (three of them with multiple drug allergy), and in three of seven patients with allergy to the remaining miscellaneous group of drugs.

In all groups of drug allergic patients the means of the logarithms of IgE values (but not the arithmetic values) were significantly different from normal (Table 1). All the nineteen patients with high serum IgE levels (not including those with allergy to inhalent allergens as well) are summarized in Table 2. In some patients with normal serum IgE, the reversed leucocyte reaction suggested an increase in cell-bound IgE.

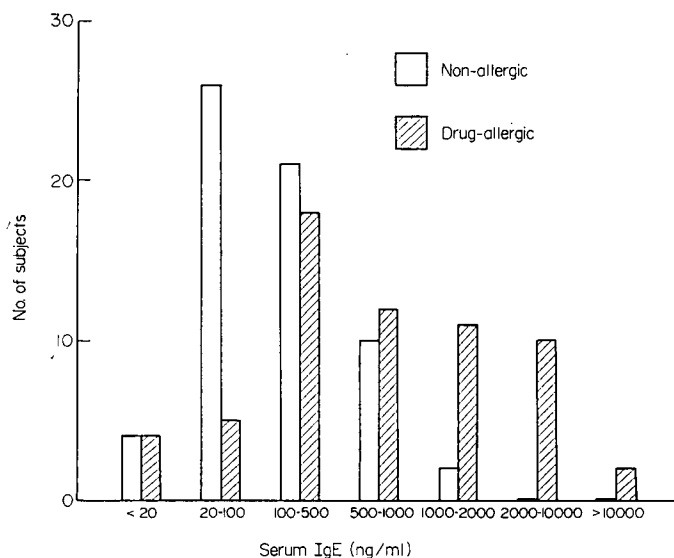


Fig. 1. Comparison of the frequency distribution of serum IgE levels in patients with drug allergy and in non-allergic controls.

Table 1. Serum IgE in normal subjects, in patients with drug allergy, and in patients with allergic asthma and rhinitis

	No.	Non-transformed values (IgE in ng/ml of serum)			Logarithmically transformed values		Statistical analysis of difference from normal*
		Range	Mean	No. of subjects with values > 900	Mean	S.E.	
Normal	63	1-1700	225	2	2.0655	0.0673	
DRUG ALLERGY							
Penicillin only	38	1-29,000	1346	9	2.4669	0.1122	0.02 < P < 0.05
Penicillin + other drugs	7	255-10,000	2357	3	2.7101	0.0488	P < 0.001
Penicillin + inhalant allergens (mainly pollen)	4	970-2250	1643	4	3.1956	0.0776	P < 0.001
Antituberculous drugs (more than one drug)	6	120-3200	1467	4	2.9031	0.2232	0.001 < P < 0.01
Other drugs (single allergy)	7	22-2332	1367	3	2.4624	0.2832	0.2 < P < 0.3
Total	62			23			
ASTHMA AND/OR RHINITIS							
House dust only (<i>D. pteronyssinus</i>)	9	4-90	40	0	1.3424	0.1365	P < 0.001†
House dust mainly	18	5-4000	585	3	1.9956	0.1679	0.7 < P < 0.8
Grass pollen only	7	53-235	123	0	2.0334	0.1001	0.8 < P < 0.9
Multiple allergy (mainly grass pollen)	16	27-20,000	2591	7	2.6532	0.2224	0.02 < P < 0.05
Total	50			10			

* Statistical analysis of difference between means was carried out by Student's *t* test.

† Serum IgE in patients with allergy to house dust only, contrary to observations with other allergens, was significantly lower than normal.

Results of other tests in drug allergy

The results of the various diagnostic procedures in patients with drug allergy are summarized in Table 3. Table 3 also shows the comparison between *in vitro* tests and the type of response to skin testing. The overall incidence of positive results was 55% in skin test, 56% in the leucocyte histamine release test, 46% in the passive lung sensitization test and 88% in the lymphocyte stimulation test. Patients with raised serum IgE had a positive skin test in 80%, a positive leucocyte test in 64%, and a positive lung sensitization test in 57%, and a positive lymphocyte stimulation test in 92%. On the other hand, in patients with normal serum IgE these tests were positive in 40%, 40%, 50% and 85% respectively.

Comparison with respiratory allergy

In fifty patients with allergic asthma and/or rhinitis serum IgE was abnormally high in only ten patients. However, all fifty patients had positive skin tests. Of these patients,

Table 2. Summary of the patients with drug allergy and raised serum IgE

Patient	Drug	Clinical manifestations	Immediate response in skin test	Histamine release from leucocytes	Passive sensitization of human lung	Lymphocyte transformation	Serum IgE (ng/ml)	
PENICILLIN ONLY								
1		Urticaria	-ve				1800	
2		Anaphylaxis, urticaria	+ve		+ve		2700	
3		Hyperpyrexia	-ve	+ve		+ve	1000	
4		Papular rash, urticaria	+ve		+ve		1600	
5		Papular rash, urticaria	+ve		-ve		1200	
6		Papular rash, urticaria	+ve		+ve		2500	
7		Anaphylaxis	+ve	+ve	+ve	+ve	29500	
8		Maculopapular rash, hepatosplenomegaly	+ve				1200	
9		Urticaria	-ve	-ve	-ve		1000	
PENICILLIN AND OTHER DRUGS								
10	PN, tetracycline	Urticaria	-ve	-ve	-ve		3400	
11	PN, Aspirin	Serum sickness syndrome	+ve	+ve		+ve	2050	
12	PN, cephaloridine, pethidine	Anaphylaxis	-ve	+ve		+ve	10000	
ANTITUBERCULOUS DRUGS								
13	Pyrazinamide Streptomycin, PAS Rifampicin Cycloserine	Hepatitis-like condition, exfoliative dermatitis		+ve	+ve	+ve	3200	
				-ve	-ve	+ve		
				-ve	-ve	+ve		
				-ve	-ve	+ve		
14	Streptomycin PAS INAH	Urticaria, fever, hepatitis-like condition	-ve	-ve	-ve	+ve	1650	
			+ve	-ve	-ve	+ve		
			-ve	-ve	-ve	+ve		
15	Rifampicin	Hepatitis-like condition		-ve	-ve	-ve	1600	
16	Streptomycin PAS		+ve	+ve				1900
MISCELLANEOUS DRUGS								
17	Aspirin	Asthma, angioedema	+ve			+ve	2332	
18	Griseofulvin	Erythema nodosum					1200	
19	Iodinated radio-contrast agents	Anaphylaxis	+ve	+ve		-ve	1500	

Table 3. Comparison of the results of various *in vitro* tests with the response to skin testing in patients with drug allergy

	Response to skin test				Incidence in all patients tested
	Immediate only	Delayed only	Immediate + delayed	Negative	
No. of patients	18 (30%)	4 (7%)	11 (18%)	27 (45%)	55 (33/60)
Per cent with raised serum IgE	56	25	64	19	37 (23/62)
Per cent with positive leucocyte test	70	100	100	12	56 (20/36)
Per cent with positive passive lung sensitization test	50	100	93	12	46 (18/39)
Per cent with positive lymphocyte stimulation test	100	100	100	66	88 (23/26)

forty-three were tested for leucocyte histamine release and for serum reagins by passive sensitization of human lung. These tests were positive in forty-one (95%) and forty-three (100%) of cases respectively. In this group, fifteen of eighteen patients (83%) had a positive lymphocyte stimulation test.

The degree of rise in serum IgE in respiratory allergy was comparable with that in penicillin allergy. Although the incidence of positive skin tests in drug allergy was smaller than in respiratory allergy, the response in some patients with drug allergy (particularly in penicillin allergy) was as strong as that in patients with extreme respiratory allergy. On the other hand, histamine release from leucocytes and from passively sensitized human lung was much higher in patients with respiratory allergy (reaching up to 100% and 70% of total leucocyte and lung tissue histamine content, respectively) than in patients with drug allergy (reaching up to 50% and 27% respectively). The incorporation of ³H-thymidine by allergen-stimulated lymphocytes reached up to 80 times the control values in drug allergy and up to fifteen-fold in respiratory allergy.

Discussion

High serum IgE levels, i.e. values above twice the standard deviation of the mean of non-allergic controls, were found in a higher proportion of subjects with drug allergy (37%) than those with extrinsic asthma and rhinitis. In all groups of patients with drug allergy the logarithmic means of serum IgE were significantly higher than normal while in patients with respiratory allergy only those with multiple allergy had a significantly higher mean. The incidence of raised IgE in the latter group was surprisingly small, and contrasts with previous studies. In one such study, Johansson (1967) found an increased serum IgE in 63% of patients with skin test positive extrinsic asthma. The low IgE levels in several patients in our series may be due to the predominance of house dust allergy. Normal or low IgE levels have in fact been reported in patients with this allergy (Berg & Johansson, 1969). In nine patients with house dust allergy, not associated with other allergies, the mean of logarithms of serum IgE was significantly lower than in non-allergic controls. This was surprising in view of the severity of clinical manifestations and the strongly positive tests with *D. pteronyssinus* extract, and the perennial symptoms. By comparison, in seven patients with pollen allergy alone, mean pre-season serum IgE was not significantly different from the normal mean.

All these values have been obtained in patients who had no hyposensitization therapy. The reversed leucocyte reaction in these patients (Assem & McAllen, 1970) suggests a discrepancy between cell-bound and serum IgE levels.

Not only was the mean level of IgE in patients with drug allergy significantly increased, but some patients showed exceptionally high levels. For example, one patient with anaphylaxis to penicillin had a level of IgE of 29,000 ng/ml. To our knowledge, previous studies, generally carried out on smaller numbers of patients, have not shown this elevation of serum IgE in drug allergy (Juhlin *et al.*, 1969).

Turning to the diagnostic value of various tests for the detection of drug allergy our results suggest that an elevated total serum IgE can be considered as supporting the diagnosis, although an absence of elevation does not disprove it. It should be added that some of our patients were investigated a long time after their allergic drug reaction, and it is possible that higher levels of IgE might have been obtained had we collected the blood samples at the height of this reaction or soon afterwards.

Although the raised IgE levels in patients with drug allergy seem best explained by their allergy, it is possible that in some patients the underlying disease, or the pathological condition resulting from the allergic reaction, might be at least a contributing factor, since it has been shown by Heiner & Rose (1970) that elevation of IgE levels may be caused by conditions other than classical allergy (e.g. liver disease).

The patients included in Table 2 illustrate some of the clinical problems related to suspected drug allergy, which may be solved by *in vitro* testing. An example is shown by the five patients developing liver disease whilst receiving antituberculous drugs such as rifampicin and isoniazid. Three of these patients have previously been reported in detail (Assem *et al.*, 1969; Hollins & Simmons, 1970).

The specific *in vitro* tests for immediate-type allergy used in our study (histamine release from leucocytes and detection of reagins by passive lung sensitization) have an established diagnostic value. They are generally positive in pollen and house dust allergy and are of much greater diagnostic value in these conditions than total serum IgE determinations. On the other hand, in penicillin allergy these tests are frequently (in about 50% of cases) negative even when pencilloyl conjugates are used (see also Assem & Schild, 1968) and diagnostically not superior to skin tests apart from the consideration of safety. The reason for the relatively frequent negative results even in established cases of penicillin allergy is probably the choice of an adequate antigen. When better test reagents are used (e.g. pencilloyl conjugates to other carrier proteins), those *in vitro* tests emerge as more sensitive and more reliable than skin tests (Assem & Vickers, in preparation).

The test of histamine release from leucocytes which detects active sensitization has a higher incidence of positive results in drug allergy than the lung test which is based on passive sensitization. It is, however, more likely to give false positive reactions to drugs causing a release of histamine which is not immunologically mediated but is due to a direct action of the drug. Thus some drugs, such as morphine, d-tubocurarine and pentamidine, may produce the release of pharmacologically active substances such as histamine, without the mediation of an immunological mechanism (see review by Paton, 1957).

The lymphocyte stimulation test gives fewer negative results than the previously mentioned *in vitro* correlates of immediate-type allergy. This is not surprising since the allergic drug reaction of most patients is likely to be due to a combination of various immunological mechanisms (immediate-type or Type I, delayed-type or Type IV, etc.;

Gell & Coombs classification, 1963). Another reason is the frequent ability of the drug itself to produce lymphocyte stimulation, while in immediate-type allergy the use of appropriate drug conjugates is of critical value. The ability of the drug itself to stimulate the incorporation of ^3H -thymidine by the lymphocytes of allergic patients is presumably due to the formation of conjugates in the lymphocyte culture. De Weck (1971) suggested that penicillin reacts with the membrane of cells in culture, and is presented in this way to antigen-sensitive cells.

In patients with penicillin allergy negative results were frequently (over 60%) obtained with the drug itself in tests for immediate-type allergy, but not in the lymphocyte stimulation test (less than 40%). This may lead to misinterpretation of other tests. For example, when these negative results coexist with a positive lymphocyte stimulation test, they suggest cell-mediated immunity. Therefore caution is needed in the interpretation of the lymphocyte stimulation test in conditions where drug conjugates have not been used.

On the whole the lymphocyte stimulation test offered little help in distinguishing the immunological mechanisms involved in drug allergy in the majority of patients; it made little difference whether they had normal or raised IgE and whether they had a late reaction in the skin test or not.

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