

Specific and total serum IgE measurements in the diagnosis of penicillin allergy. A long term follow-up study

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Summary

Sera from 204 patients with acute or former reactions following penicillin treatment were investigated by RAST with specificity for benzylpenicilloyl and phenoxymethylpenicilloyl. The IgE antibody levels were followed up for more than 2 years. Positive results could be observed at the time of the acute reactions and the weeks following; later on the amounts of specific IgE decreased, with negative RAST results after months or years in many cases. Occasionally negative RAST results occurred within 30 days; on the other hand high levels of specific IgE persisted for years in some patients. Total serum IgE measured by RIST and the RAST results showed similar patterns, with mean levels of 568 u/ml between 0 and 30 days after the penicillin allergic reaction, 286 u/ml between 30 days and 1 year, and 195 u/ml in cases after 1 year corresponded well with the observed decline in specific IgE.

Skin tests performed with penicilloyl-polylysine in 118 patients showed an overall correlation of 91.5% with the RAST results. Differences between the two test systems were observed in patients with a long interval between the test and the last allergic attack. None of the patients with proved penicillin allergy had atopic diseases or a history of atopy.

On the basis of these findings it is suggested that the determination of both circulating specific IgE and total IgE should be of great value for diagnostic purposes during acute drug reactions and in the months immediately afterwards.

Introduction

Since the discovery of IgE as the main carrier of reaginic antibody activity, atopic patients have been considered to be persons with high risk for IgE-mediated allergy after drug treatment (Idsoe *et al.*, 1968). Patients with acute drug reactions have usually shown higher total IgE values than normal individuals (Assem & Vickers, 1972; Brehm, 1974) but in longitudinal studies the non-specific total IgE has had a tendency to fall to normal levels after disappearance of clinical symptoms (Müller, Morell & Hoigné, 1973). This implies that non-atopic patients are also capable of forming

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IgE antibodies against drugs, but this has yet to be substantiated by specific IgE determinations.

Penicilloyl-specific IgE can be measured by RAST and a direct correlation has been found between specific IgE levels and positive skin tests (Wide & Juhlin, 1971; Juhlin & Wide, 1972; Adkinson, 1975) which are thought to be predictive of immediate type reactions to penicillin (Parker *et al.*, 1962; de Weck & Blum, 1965; Levine & Zolov, 1969). Previous results indicate that the RAST is a valuable alternative to the more dangerous and time consuming skin tests and may aid early diagnosis of penicillin allergy (Kraft & Wide, 1976).

In the present investigation two aspects were of interest. Firstly, it was important to know at what stage of the allergic reaction and for how long after the allergic reaction positive results can be expected in RAST. The second aspect was to search for an atopic background of patients with specific IgE. The investigation was designed to determine whether or not those patients producing high specific IgE levels were atopic or if other factors had to be considered. Therefore the levels of specific IgE antibodies and total IgE in penicillin allergic patients were followed up for more than 2 years and the history of atopy in RAST-positive cases was investigated in detail.

Patients

Two hundred and four patients (ninety-six females, 108 males) aged from 1·3 to 83 years (average 43·1) and with suspected penicillin allergy were included in the study. They had exhibited clinical symptoms after treatment with different penicillins (sodium penicillin G, procaine penicillin G, clemizole penicillin G, penicillin V, cloxacillin, ciclacillin, amoxycillin, carbenicillin and ampicillin) either in the 2nd Department of Dermatology, University of Vienna, or during consultant visits to other University or City hospitals in Vienna. The patients were divided into four groups according to the date of the drug reaction and the performance of diagnostic tests:

Group A

Sixty-nine patients who had been examined within the first 2 days of acute reactions to penicillin and who were tested for circulating total IgE and specific IgE and by skin tests.

Group B

Forty-nine patients with a history of reactions to penicillin in the period 3 weeks–5 years before the study and who were tested for circulating total IgE and specific IgE and by skin tests.

Group C

Seventy-six patients who were examined during the first 2 days of acute reactions to penicillin but tested by *in vitro* tests only.

Group D

Ten patients who had exhibited penicillin allergy which was proved by skin tests in the period 2–5 years before the study and who were tested by *in vitro* tests.

Materials and methods

Sera

Blood samples were drawn from each patient at the time of the drug reaction and/or

on several occasions afterwards. Additional samples were obtained from patients of group A and B at the time of skin testing. After clotting at room temperature for 2 hr the sera were separated and stored at -25°C until the *in vitro* tests were performed.

Circulating IgE antibodies

IgE antibodies to penicillin in the serum were assayed in duplicate by the radio-allergosorbent test, RAST (Wide, Bennich & Johansson, 1967). Benzylpenicilloyl-human serum albumin (BPO) and phenoxymethylpenicilloyl-human serum albumin (PMPO) respectively were coupled to cyanogen-bromide-activated cellulose paper discs (RAST[®], Pharmacia Diagnostics AB, Uppsala, Sweden). The RAST results were expressed in Phadebas RAST classes 0, 1, 2, 3 and 4 according to a reference system established by running four reference sera (A, B, C and D) with reference discs during each assay in parallel to the unknown samples. In this study class 0 was considered to be a negative RAST result. The test was performed according to instructions for use given by the manufacturer. Stability controls of the antigen discs were established by repeated assays of sera 2 years after the initial testing.

Serum IgE levels

The determination of the IgE concentration in the sera was made with Phadebas[®] IgE test (Pharmacia Diagnostics AB, Uppsala, Sweden) which is based on the radio-immunosorbent technique, RIST (Wide, Axén & Porath, 1967) as applied to the assay of IgE (Johansson, Bennich & Wide, 1968).

Skin tests

The tests were performed with different antigens and different concentrations on the inner side of the forearm as reported in detail elsewhere (Kraft & Wide, 1976). Benzylpenicilloyl-polylysine (PPL)*, benzylpenicilloic acid* and ampicillin (Binotal) were used for scratch and intradermal tests. The diameters of weal and flare reactions were measured after 20 min and the reaction was considered positive when the diameter was greater than 5 mm. Controls with isotonic NaCl solution and in doubtful cases with a histamine solution (10 mg/ml for scratch and 0.1 mg/ml for intradermal tests) were used.

Results

Clinical data

The observed clinical patterns of adverse reactions to penicillin are recorded in Table 1. Skin eruptions like urticaria (eighty-three) and scarlatiniform or morbilliform exanthems (fifty-one) were seen most frequently. Polymorphic exanthems (thirty-seven), characterized by a variety of different lesions (large macular, maculopapulovesicular and purpuric) at the same time, were next in frequency followed by anaphylactic shock (twenty-two) and rare manifestations such as drug fever, fixed drug eruption or Arthus-reaction at the injection site. None of the patients suffered from atopic diseases or had a history of atopy.

* The test substances were kindly supplied by Professor Dr A. L. de Weck, Bern, and Hoffmann La Roche, Basel.

Table 1. Clinical patterns in relation to different patient groups (A, B, C and D)

Drug reaction	A	B	C	D	Total
Anaphylactic shock	3 (2)	5 (2)	12 (7)	2 (1)	22 (12)
Urticaria	38 (17)	24 (7)	15 (9)	6 (2)	83 (35)
Serum sickness-like reactions	3 (0)	1 (1)			4 (1)
Scarlatiniform or morbilliform exanthem	12 (0)	6 (0)	33 (0)		51 (0)
Polymorphic exanthem	10 (1)	10 (4)	15 (5)	2 (1)	37 (11)
Drug fever	1 (0)				1 (0)
Fixed drug eruption	2 (0)				2 (0)
Arthus-type reaction (injection site)		1 (0)			1 (0)
Questionable		2 (0)	1 (1)		3 (1)
Total	69 (20)	49 (14)	76 (22)	10 (4)	204 (60)

() positive in RAST.

Positive RAST-results and different clinical patterns

Forty-seven (78.3%) of sixty patients with positive results in RAST had clinical symptoms of anaphylactic shock and urticaria (Table 1). Specific IgE was also found in eleven cases of polymorphic exanthems (18.3%) but neither in patients with scarlatiniform or morbilliform exanthems, nor with drug fever or fixed drug eruptions.

Incidence and strength of positive RAST-results in patients with immediate type reactions

Thirty-five (51.5%) of sixty-eight patients with immediate type reactions (anaphylactic shock and urticaria) at the time of study (groups A and C) showed positive RAST results when tested during the drug reaction or in the first 30 days (Table 1). In sixteen of these patients the specific IgE activity corresponded to RAST classes 3 and 4. In patients with a history of immediate type reactions to penicillin which was not so recent (group B) nine (31.0%) out of twenty-nine exhibited circulating specific IgE, three of which were related to RAST classes higher than 2. Four of ten patients, with penicillin allergy established 1–5 years previously (group D), had circulating IgE antibodies but in a fairly low concentration (RAST—classes 1 and 2).

In five of sixty cases the BPO-specific RAST detected levels of IgE-antibodies two classes higher than in the PMPO-specific test. Differences of one class were found in relation to both BPO and PMPO, thus emphasizing the importance of using more than one antigenic determinant.

Agreement between RAST and skin test results

Skin tests could be performed in 118 of the 204 patients and positive results within 20 min were obtained with PPL in thirty-five cases (Table 2). Ten patients (three in group A and seven in group B) with positive skin tests to PPL had negative RAST whereas none of the RAST positive patients had negative skin tests. The overall agreement between the two tests was 95.7% for group A, 85.7% for group B and 91.5% for the two groups combined.

Skin tests performed with other antigenic determinants such as sodium penicillin

Table 2. Correlation of RAST and skin test results in penicillin allergy

Skin tests (performed with PPL)	RAST (BPO- and PMPO-specific)			
	Group A (69 patients)		Group B (79 patients)	
	+	-	+	-
+	16	3	9	7
-	0	50	0	33
Agreement	95.7%		85.7%	

G, penicilloic acid and ampicillin gave additional information in twelve (10.2%) patients who were negative in the RAST.

In group D, four of ten patients with a history of penicillin allergy (proved by skin tests 2-5 years previously) showed a positive RAST.

Prolonged study of specific IgE levels

Thirty-two out of forty-five patients, with positive RAST results at the time of the acute drug reaction or in the first thirty day period, were followed up and the initial RAST compared to later RAST results (Fig. 1-I, blank columns). In nineteen out of twenty-one patients (Fig. 1-II) a decrease of specific IgE activity, corresponding to lower RAST classes, was observed during the first year, and five cases became negative. In the remaining two patients a small increase of specific IgE antibodies

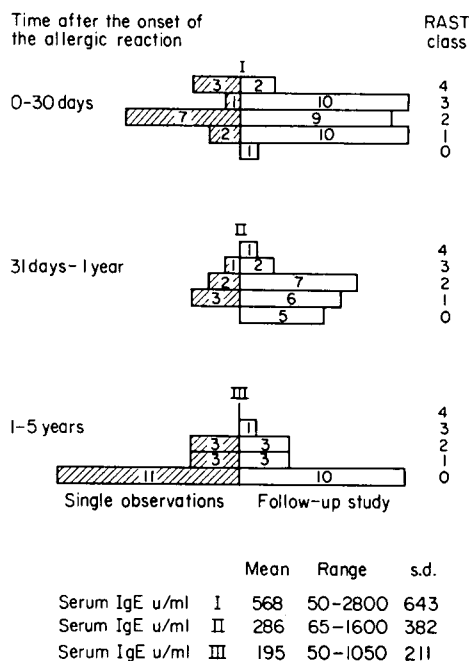


Fig. 1. Changes of serum concentration of IgE antibodies and serum IgE in penicillin allergic patients. The numbers in the histograms indicate the numbers of patients.

(classes 0-1 and 3-4) was found. Of sera of seventeen patients tested 1-5 years after the allergic reaction, four were in the same RAST-classes as found initially (classes 2 and 3), whereas ten patients showed negative results (Fig. 1-III). Twenty-three patients with single observations at different time intervals after the allergic reaction (Fig. 1-II and III, hatched columns) fitted well into this pattern.

Total IgE levels

In patients with acute penicillin allergy the total IgE levels measured by RIST were found to be significantly higher during the first 30 days (mean 568 u/ml) than during the following 11 months ($P < 0.001$, mean 286 u/ml) or even years ($P < 0.001$, mean 195 u/ml). One to five years after the allergic reaction RAST results of classes 2 and 3 were reflected by serum IgE levels between 110 and 340 u/ml. Two patients (classes 0 and 1 in RAST) showed unusually high IgE levels (880 and 1050 u/ml) but there was no evidence for atopic disease or history of atopy.

Discussion

In the present study previously reported results were confirmed and the study was extended so that the RAST could provide an early diagnosis of penicillin allergy as previously considered (Kraft & Wide, 1976). The strongest and most frequently observed positive RAST results were found at the time of the allergic reaction or within the next 30 days. It seems that during the allergic attack enough specific IgE is in the circulation and available in serum for positive *in vitro* estimation and/or there is a subsequent rapid production of IgE (half life 2-3 days). On the other hand, a clear decline of specific IgE with time was observed in thirty-two patients in the follow-up study; five of twenty-one tested had negative results in RAST after 1-12 months, and ten of seventeen tested showed negative results after more than 1 year. The same pattern was found in twenty-three patients with single observations only. The conclusion to be drawn from the investigations therefore would be that RAST should be used in all cases where a quick diagnosis is necessary, e.g. in intensive care units or with patients who are undergoing hospital treatment as a result of an acute allergic attack. If the RAST is negative at the time of the drug reaction, it should be repeated within the next few weeks to avoid a false negative diagnosis, as was the case with one patient. The later fall of specific IgE may limit the applicability of RAST or other *in vitro* tests and should be considered when patients, with a history of reactions to penicillin, are tested. The same limitation also applies to skin tests (Finke *et al.*, 1965). It is interesting to note that using a venom-specific RAST similar patterns of specific IgE were observed in patients with allergic reactions to stinging insects (Light, Reisman & Arbesman, 1975; Yunginger & Gleich, 1975) and some parallels in the immune response can be assumed.

The high levels of total serum IgE at the time of the allergic reaction and their subsequent decrease corresponded well with the observed changes in specific IgE. Patients with penicillin allergy usually showed normal or, in a few cases, elevated total IgE after 1 or more years. Neither atopic diseases nor a history of atopy could be found in any of these patients. The persistence of specific IgE is certainly not related to atopy (Stember & Levine, 1973). Other genetic factors like Ir-genes, causing recognition of minute doses of penicillin in the environment (in food or produced by fungi after infection), have also to be considered. Those patients, who have high levels of specific IgE, remain in our opinion clinically at risk for drug induced type I re-

actions. This was seen, for example, in a patient with a high level of circulating BPO-specific IgE and anaphylactic shock after carefully performed skin testing (Kraft & Wide, 1976). Three years afterwards this patient still showed a high level of specific IgE (RAST—class 3).

Positive skin tests of the immediate type are predictive of type I reactions (Levine & Zolov, 1969; Parker, 1970; McGovern, Roberson & Stewart, 1970; de Weck, 1972). In our study an overall correlation of 91.5% was found between the results of skin tests and RAST. As shown before, the RAST seems to be a very valuable *in vitro* test for detection of patients at risk for type I reactions. The correlation between the results of the two test systems was 95.7% for group A (acute reactions) and 85.7% for group B (patients with case history). These results also indicate the usefulness of testing as early as possible after suspected adverse reaction to penicillin and of repetition of the tests if negative results are obtained. Disagreement was found in ten patients with PPL-positive skin tests and negative RAST results. The presence of short term anaphylactic antibodies (Parish, 1970 and 1974) may explain such discrepancies in some cases but no conclusions have been drawn about their relationship to the IgG₄-subclass in penicillin allergy (Kraft, Wilson & Devey, 1976).

The above reported correlations were obtained in 118 patients on the basis of penicilloyl-specificity in the skin tests. The use of additional antigens such as penicillin G, penicilloic acid and ampicillin gave more information in twelve cases (10.2%) where skin tests with PPL and the RAST were negative. Therefore additional antigenic determinants in all test systems may give more detailed and more comprehensive information (de Weck, 1972; Schneider, 1974).

In summary, the RAST seems to have sufficient sensitivity to detect patients at high risk for IgE-mediated drug reactions, especially when tests are performed during the first weeks or at least in the first year after a suspected drug induced allergic reaction.

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