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RAST-based immunotherapy

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For more than half a century immunotherapy has been an important cornerstone in the management of allergic rhinitis. The usefulness of this form of therapy has been documented in several controlled studies which have shown that successful results occur on the basis of definite immunologic changes that are dose-related (Johnstone, 1957; Frankland and Augustin, 1954; Van Metre et al., 1980); that is, in order to obtain significant relief from symptoms, patients usually require the administration of high doses of the specific offending allergen. A limiting factor in achieving such effective dosage, however, is the threat of causing undesirable systemic reactions (Van Arsdel and Sherman, 1957). It has been observed that these reactions occur at a greater frequency and severity in those patients with high serum levels of skin-sensitizing antibody (allergen-specific IgE) as measured by the Prausnitz-Kustner (PK) transfer technique. It has also been reported that an inverse relationship exists between the highest tolerated dose and this serum antibody titer (Connell et al., 1957; 1962; 1964;). In 1967, with the development of the radioallergosorbent (RAST) test for the detection of specific IgE antibody levels in serum, a great advance was made in the field of allergology (Wide et al., 1967). For the first time physicians had available a relatively simple test for the detection of specific-IgE antibody in serum and the in-vitro estimation of these titers without the problems inherent in performing PK transfers. This report describes a technique in which individual sensitivity to clinically-relevant allergens is determined by a modified version of the RAST procedure and the information used by the physician as a guide in selecting safe initial dose levels to be given in the immunotherapy for allergic rhinitis. We have found that by employing this information untoward reactions are predictable and therefore, avoidable. The net effect of incorporating the direct measurement of specific-IgE antibody into the decision making in allergy practice has been that fewer patients are started on immunotherapy, initial treatment doses are administered at higher levels than previously considered possible, and physicians have been alerted to those situations in which the patient is at risk for having an adverse reaction.

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MODIFIED RAST PROCEDURE FOR DETECTION OF SPECIFIC IgE

In the RAST allergens are chemically linked to solid-phase supports, usually paper discs, which are incubated with a droplet of serum from the allergic patient. If specific-IgE antibodies are present in the serum sample they will bind with the insolubilized allergen to form a complex. After this first step, the allergen-coated paper discs are washed to remove all of the unattached serum protein. The allergen-IgE antibody complexs (the discs) are then incubated with labeled anti-human IgE antibodies which have been raised in another species usually the rabbit.

After this second step, all free labeled anti-IgE is washed away; the more specific IgE initially bound to the solid phase, the more rabbit anti-IgE with its attached radioactive tag left behind to be counted in a scintillation gamma counter.

Since 1977, we have utilized a modification, described in more detail elsewhere, of the commercially available RAST (Phadebas RAST, Pharmacia Diagnostics) (Nalebuff et al., 1981). This Modified RAST (MRT) has been shown to have increased test sensitivity without a significant loss of specificity (De Filippi et al., 1981; Nalebuff, 1981). This was accomplished by extending the initial incubation period from three to eighteen hours and increasing the volume of serum under test from 50 to 100 µl. After the second incubation period with the labeled-rabbit antibody against human IgE, and prior to counting the disc-bound radioactivity, an additional step must be performed; the allergen coated discs are removed from their original tubes and placed into fresh ones to insure that only radioactivity immunologically bound to them is measured. It can be shown that despite careful washing a small amount of radioactivity adheres to the inner surface of the carrier polystyrene test tube; this can take on significance in those sera with low levels obtained with the negative controls (Santrach et al., 1981).

SCORING SYSTEM

In this system the lower limit of detectable levels of allergen-specific IgE averages 1.5 times the binding of negative controls consisting of either human cord serum, serum from non-atopic patients or from highly atopic patients tested against an inappropriate allergen disc. The average non-specific binding in the system averages 500 counts (Table 1). Seven hundred and fifty counts in the MRT scoring system is at the ninety-five confidence level for the detection of specific IgE antibody (Hoffman, 1980). The observed binding in this system ranges from 1.5 to 80 times that of the non-specific background levels and for convenience has been divided into five distinct classes. Each class represents approximately a five-fold increase in the amount of serum specific IgE antibody. Class 1 (750–1600 counts) is considered to be an equivocal score in which IgE antibody is detectable but may not be clinically-relevant. Although class 1 levels are usually associated with a positive skin test reaction, only about half will respond when suitably chal-

Table 1. The modified rast scoring system.

MRT class*	counts**	interpretation
0	250–750	Below detection
1	750–1600	Equivocal
2	1600–3600	Positive with
3	3600–8000	increasing levels of
4	8000–18000	allergen-specific
5	18000–40000	IgE antibody

* Each class represents a five-fold increase in detectable specific IgE antibody.
** These are the counts obtained when a positive control sera (25 U IgE) gives 25,000 counts. Non-specific binding of the negative control averages 500 counts for each allergen.

lenged. Class 2 scores and above (1600–40000 counts) represent positive scores with increasing levels of detectable specific-IgE antibody and degrees of clinical sensitivity. Ninety-five percent of patients with these scores will promptly respond to a properly performed nasal of conjunctival provocation test (Santrach et al., 1981).

SELECTION OF INITIAL DOSE

The recommended initial immunotherapy doses are given inversely proportional to each MRT score. Patients are regularly started at allergen concentration of 1 : 500 w/v for those allergens with low serum specific IgE levels (class 1). As the serum antibody concentration rises, the suggested initial dose is proportionally decreased in five-fold increments. For those allergens in which the specific-antibody score is extremely high (class 5) the recommended initial doses is significantly lower at an allergen concentration of 1 : 312,000 w/v (Table 2). After the initial doses have been administered and tolerated injections are usually doubled

Table 2. Distribution of initial immunotherapy doses.

		dose schedule (w/v)	
MRT class	percentage MRT tests	regular	aggressive
0	50	–	–
1	25	1 : 500	1 : 100
2	12	1 : 2,500	1 : 500
3	6	1 : 12,500	1 : 2,500
4	4	1 : 62,500	1 : 12,500
5	3	1 : 312,500	1 : 62,500

As a safety precaution, the initial injection should be given intracutaneously as a skin test challenge to produce a 4 mm wheal and the patient carefully observed. The dose is considered correct if the wheal size is 15 mm or less after 10 minutes. If no adverse reaction occurs, the dose can safely be given subcutaneously.

at each subsequent visit, unless the clinical response suggests it would not be safe, until the dose nears the customary maximum level. Because of standardization problems associated with the manufacture of allergen extracts, an exact optimum dose level is difficult to define (concentrations ranging from 1 : 20 to 1 : 5000 w/v have been reported to be effective) (Johnstone, 1957).

SAFETY PRECAUTION

Prior to starting immunotherapy, it is mandatory that a small amount of the incriminated allergen be placed intradermally as a skin-test challenge (enough of the allergen must be injected to produce a 4 mm skin wheal). The patient is then observed and if in ten minutes this in-vivo challenge produces a wheal of 15 mm or less, the suggested dose is given subcutaneously. While this challenge is performed in all patients, it is especially important for class 1 thru class 3 allergens where the suggested RAST-based doses are relatively high (1 : 500 to 12500 w/v). With class 4 and 5 allergens, the in-vivo challenges are less important because the suggested allergen doses (1 : 62,500 to 1 : 312,000 w/v) are consistent with levels considered safe even for extremely sensitive patients (Van Metre et al., 1980). Nevertheless, even in these situations, the in-vivo challenge serves the useful purpose of confirming the biological potency of the highly diluted allergen extract. Only rarely, in our experience, have the skin test challenge responses suggested that the Rast-based dose would be extensive.

RESULTS

A review of the MRT records of over a thousand rhinitis patients treated in our clinic between 1979–1982 revealed that on an average they were sensitive to ten allergens; this represents about fifty percent of the tests. Eight-five percent of the tests with detectable levels had MRT scores at the lower levels (classes one through three) as shown in Table 2; these allergens were considered unlikely to cause a constitutional reaction in therapy and initial treatment doses for these were administered after the preliminary skin test challenge, at the regular suggested doses (Table 2) (Nalebuff and Fadal, 1980).

All patients not only tolerated their initial high doses of these allergens, but were readily advanced to doses containing 1000 to 2500 Noon units; the equivalent of 0.1 to 0.25 ml of a 1 : 100 w/v concentration of the allergen material. In these same patients, the remaining fifteen percent of incriminated allergens were associated with high levels of specific-IgE antibody (class 4 and class 5 MRT scores) and were started in therapy at the regular lower dose levels usually used to initiate such treatment. These allergens were also advanced on a more deliberate schedule. We were not able to routinely raise these allergens to the high levels achievable with the others in these same patients. They commonly caused large local and even mild constitutional reactions when high dosage levels were attempted –

often after a years course of allergen immunotherapy. During the past six years, the only constitutional reactions requiring medical intervention have occurred with those allergens associated with initially high MRT scores in whom we attempted to raise the dose above the 1 : 500 w/v concentration.

In a previously published paper on the effects of RAST-based immunotherapy in forty-six allergic rhinitis patients in whom pharmacological agents had failed to provide relief, we noted that 41/46 patients obtained symptomatic improvement within 24 weeks (Fadal and Nalebuff, 1980). In another study, two-hundred ragweed-sensitive hayfever patients were also started on treatment with initial doses suggested by their MRT score (Nalebuff et al., 1981). These patients all had positive histories of seasonal rhinitis occurring from August through October, demonstrated skin reactivity of 3+ or more with a short ragweed extract at a positive nasal or conjunctival provocation upon sequential challenge with the ragweed pollen extract at a concentration of 1 : 100 w/v or less. Ninety serums were scored as MRT class one for the ragweed allergen and these patients were given high doses of the extract. In order to stress the system, the doses employed were one five-fold level more concentrated then regularly used that is, the aggressive schedule listed in Table 2 was followed. These patients were given, as their initial dose, 0.1 ml of a 1 : 100 w/v material containing 3 µg of Antigen E (AgE); no systemic reactions occurred. One-third of these patients did experience large local reactions which was attributed to the high concentration of glycerin remaining in the extract at the selected concentration. Fifty-two patients were started on therapy with allergen material at a 1 : 500 w/v concentration. Only six patients were started at the lowest dose used in this particular study, a concentration of 1 : 62000 w/v. Despite the fact that seventy percent of these patients (140/200) received 3 µg doses of AgE within the first week of treatment, no constitutional reactions occurred the entire group.

Similar findings were reported by Young (1981) in a study to determine whether the MRT score could safely predict a safe initial starting dose. His study included forty patients with a history of rhinitis with a seasonal exacerbation to either timothy or ragweed pollen. All patients had a 2+ or greater skin reaction to the study antigens. Twenty-six of these patients were in class 1 through 3 and given relatively high doses suggested by their serum levels of IgE antibody. Fourteen patients were started a modest dose because of the higher serum levels; all patients were able to tolerate the initial dose without reaction.

A similar observation was reported by Kane et al. among thirty-six ragweed sensitive patients (Kane et al., 1981). Four additional skin test positive patients, considered ill enough to warrant immunotherapy, had negative MRT scores and were not included. Eight of the thirty-six challenged patients had MRT class one scores and were stated at a dose level of allergen at a 1 : 500 w/v concentration. All thirty-six patients tolerated the initial doses which ranged from 1 : 500 w/v to

1 : 62500 w/v. Unfortunately the allergen material utilized was weak and, therefore, did not adequately stress the system.

In a study reported by Santrach et al. (1981), fifty patients were started on immunotherapy with initial doses based on the MRT score. All thirty-five patients with class 1 to class 3 scores were given their initial doses according to the more aggressive dose schedule (1 : 100 to 1 : 2500 w/v) and tolerated the dose without incident. The initial doses were withheld, however, in fifteen other patients because the preliminary the skin test challenge gave greater than a 15 mm wheal. Thus, these investigators reported that thirty-percent (15/50) of their patients could not accept the suggested MRT dose; even when the skin-test challenge was done at the regular levels suggested in Table 2, five patients still failed the preliminary skin test challenge.

Examination of the data from these patients shows that the recommended doses were at a 1 : 312.000 w/v concentration – a level considered safe by most allergists even for their highly sensitive patients (Van Metre et al., 1980). In fact, Santrach and associates even noted that the initial doses in the MRT system for such patients are actually less that recommended by most traditional allergists for such patients. Thus it is likely that those patients who did not pass the skin test challenge could have easily tolerated the suggestive initial dose.

DISCUSSION

In the mid-1950s two double-blind controlled studies were reported which confirmed the effectiveness of immunotherapy in the management of allergic patients. In the first study by Johnstone (1957), 105 patients were treated with immunotherapy with allergen doses gradually raised to concentrations of either 1 : 500 w/v (high dose) or 1 : 5000 w/v (moderately high dose). A second group of 105 patients was given placebo therapy with either a saline injection or the appropriate allergen at a concentration of 1 : 10,000,000 w/v. Significant improvement in symptoms scores and a diminution in the incidence and subsequent development of asthma, was noted in the actively treated group. A similar study by Frankland and Augustin (1954) involved two hundred patients. One-half were treated with allergen while the other half were given placebo therapy. A marked improvement was noted only in the treated group in whom doses were raised to the level of 1 : 50 w/v. Despite the excellent results reported, it was noted that thirty percent of the treated patients experienced systemic reactions. As one would expect, there were no such reactions noted in the placebo treated group. In a more recent study by Van Metre et al. (1980) low doses failed to lead to the same objective immune responses obtained with the high doses customarily recommended in immunotherapy regimens. In twelve patients treated for Ragweed hayfever in whom final doses of 2.7 μg of AgE or greater were reached, there were marked increases in the measured levels of IgG blocking antibody.

No such findings were observed among twelve similar patients in whom the doses were kept at a significantly lower level. In a retrospective report by Van Arsdale and Sherman, the case records of 8706 patients were reviewed. These patients had 1774 constitutional reactions during their immunotherapy; it was observed that all of these reactions occurred in 663 patients. Particularly significant was the fact that 351 (4%) of these patients had 1462 (82%) of the adverse reactions. In attempt to identify those patients at risk, Connell and Sherman (1964) measured the skin-sensitizing antibody titer in 69 patients treated with an acqueous extract of ragweed pollen; in this group the titers ranged from ten to over a thousand. Thirteen of these patients experienced systemic reactions. They noted that all of the untoward reactions occurred in the thirty-two patients with titers over 100; ten of them in patients with SSA titers over 500. Similar findings occurred when the allergen was changed to a ragweed emulsion. Nine constitutional reactions occurred in 85 patients so treated; eight of these in patients with SSA titers of 500 or greater. No reactions occurred in the 18 patients whose SSA titers were less than 100. In view of these observations it was suggested by Connell and Sherman (1962) that the SSA titer might serve as the most valuable piece of information upon which to select initial doses of allergen extracts.

Since there is such a high correlation between the SSA titer and the RAST score, it is not surprising that Bernstein (1975) found that patients with high RAST scores could tolerate only the smallest amount of allergen extract; he advised that such patients be routinely started with extremely dilute material and that doses be raised cautiously.

We believe that the method of determing initial immunotherapy doses described in this report represents one of the main advantages of incorporating RAST in the management of allergic patients. High RAST scores alert the clinician to those situations where specific IgE antibodies are present at a high-enough concentration to cause systemic reactions. Low RAST scores, on the other hand, allow the physician to be more aggressive both in initiating and advancing the dosages; many such patients have marked skin reactivity which tends to over estimate sensitivity. In the Santrach study the eight patients with MRT class one scores had skin test end-points ranging from 1 : 100 to 1 : 312.000 w/v. The early administration of allergen immunotherapy at dose levels previously shown to stimulate the immune system affords the treating physician with a modality which brings a degree of science to an area of medical practice often functioning as an art form. There are many physicians, allergists included, who feel that the usual form of immunotherapy should be abandoned in view of the symptomatic relief often achieved with antihistamines, topical steroids and the like. This can best be appreciated when one compares the doses administered utilizing the RAST-based method to those suggested in traditional approaches where all initial doses are usually given at concentrations of 1 : 250,000 w/v or even lower and increased

over a period of several months; twenty-four or more injections may be required before such levels are reached (Van Metre et al., 1980). At this point it should be emphasized that the selection of patients to be started on immunotherapy and the doses finally reached must be made in conjunction with the physicians clinical judgement. The detection of allergen-specific IgE levels by the MRT is not in itself a valid indication for starting patients on immunotherapy.

Nevertheless the discovery of IgE as the immunoglobulin responsible for most reaginic activity and the development of diagnostic tests for its in-vitro measurement should continue to result in improved treatment for rhinitis patients ill enough to require allergen immunotherapy.

REFERENCES

1. Arsdal, P. van and Sherman, W. B., 1957: The risk of inducing constitutional reactions in allergic patients. J. Allergy, 28, 251.
2. Bernstein, I. L., 1975: Experience with RAST in the diagnosis and management of inhalant allergy. Advances in diagnosis of allergy: RAST (Evans, R. III ed.), Miami, Symposia Specialists, 17.
3. Connell, J. T., Sherman, W. B. and Meyers, A. P., 1957: The risks of inducing constitutional reactions in allergic patients. J. Allergy, 28, 251.
4. Connell, J. T., Sherman, W. B. and Meyers, A. P., 1962: Antibody studies in constitutional reactions resulting from injections of ragweed pollen extract. J. Allergy, 33, 365.
5. Connell, J. T., Sherman, W. B. and Meyers, A. P., 1964: Skin-sensitizing antibody titer III. Relationship of the skin-sensitizing antibody titer to the intracutaneous skin test, to the tolerance of injections of antigens, and to the effects of prolonged treatment with antigen. J. Allergy, 35, 169.
6. Fadal, R. G. and Nalebuff, D. J., 1980: A study of optimum dose immunotherapy in pharmalogical treatment failures. Arch. Otolaryngol., 106, 38.
7. Filippi, I. de, Yman, L. and Schroder, H., 1981: Clinical accuracy of updated version of the Phadebas Rast test. Ann. Allerg. 46, 249.
8. Frankland, A. W. and Augustin, R., 1954: Prophylaxis of summer hayfever and asthma: A controlled trial comparing crude grass-pollen extracts with the isolated main protein component. Lancet, 1, 1055.
9. Hoffman, D. R., 1980: Comparison of methods of performing the radioallergosorbent test: Phadebas, Fadal/Nalebuff and Hoffman Protocols. Ann. Allerg., 45, 343.
10. Johnstone, D. E., 1957: Study of the role of antigen dose in the treatment of pollinosis and pollen asthma. J. Dis. Child., 94, 1.
11. Kane, J., Wypych, J., Reisman, R. et al., 1981: Evaluation of serum RAST titers to determine initial doses for ragweed immunotherapy (abstract). Read before the 37th Annual Meeting of the Amer. Acad. Allergy, San Francisco.
12. Metre, T. E. van, Adkinson, N. F., Lichtenstein, L. M. et al., 1980: A controlled study of the effectiveness of the Rinkel method of immunotherapy for ragweed pollen hay fever. J. Allergy Clin. Imm., 65, 288.
13. Nalebuff, D. J. and Fadal, R. G., 1980: In-vitro determination of initial immunotherapy dosage: A new clinical application of the radioallergosorbent test. Allergy: Including IgE in diagnosis and treatment (Johnson, F., ed.), Miami, Symposia Specialists, 105.
14. Nalebuff, D. J., Fadal, R. G. and Ali, M., 1981: Development of the modified RAST, RAST in clinical allergy (Fadal/Nalebuff eds), Miami, Symposia Specialists, 35.
15. Nalebuff, D. J., 1981: An enthusiastic view of the use of RAST in clinical allergy. Immunology and Allergy Practice, 3, 18.

16. Nalebuff, D. J., Fadal, R. G. and Ali, M., 1981: Determination of initial immunotherapy dose for ragweed hypersensitivity with the modified RAST test. Otolaryngol. Head Neck Surg., 89, 271.
17. Santrach, P. J., Parker, J. L., Jones, R. T. and Yunginger, J. W., 1981: Diagnostic and therapeutic applications of a modified radioallergosorbent test in comparison with conventional radioallergosorbent test. J. Allergy Clin. Imm., 67, 97.
18. Wide, L., Bennich, H. and Johansson, S. G. O., 1967: Diagnosis of allergy by an in-vitro test for allergen antibodies. Lancet 2, 1105.
19. Young, S. H., 1981: Use of the modified RAST to determine initial immunotherapy dose, RAST in clinical allergy (Fadal/Nalebuff eds), Miami, Symposia Specialists, 217.

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