

Allergenicity of alum-precipitated grass pollen extracts with different RAST activity

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Summary

The biological potency of therapeutic allergen extracts is fundamentally important in terms of maximal security of immunotherapy, especially when changing batches. As regards pyridine-extracted alum-precipitated allergen preparations, the potency is commercially controlled by the RAST technique. In the present study, titrated intradermal tests were performed in thirty-three adult grass-pollen rhinitis patients with three ten-fold dilutions of two batches of Allpyral® 5-grass mix with different RAST activity. The difference in potency was estimated as the horizontal distance at the midpoint of the two dose–response curves after performing linear regression on the log-dose/log-weal area response, and as the concentration of each batch eliciting a weal equal to the histamine HCl 0.1 mg/ml reference. The difference in potency as estimated by RAST was a factor 5.0 *vs* a factor 8.3 and 7.2 (median) estimated by the horizontal distance and histamine-equivalent reaction, respectively. Considering the biological variation, the RAST-estimated difference in potency is in the same order as that found by skin test. It is, therefore, concluded that the routine control of new production batches by RAST is acceptable with the use of standardized reference sera as regards alum-precipitated grass pollen allergen extracts.

Introduction

Biological standardization of allergenic extracts is related to the allergenicity, i.e. the ability to elicit IgE-mediated reactions. The importance of estimating the allergenicity of both diagnostic and therapeutic extracts has been stressed by many authors [1–4] and is, furthermore, required by the registration authorities [5]. Concerning allergen extracts intended for immunotherapy (therapeutic extracts), standardization is fundamentally important in terms of maximal security when changing batches. As for aqueous extracts they are easily standardized and their allergenicity estimated by procedures such as skin test and RAST (total biological activity) or quantitative

immunoelectrophoresis (QIE), which expresses the qualitative composition of the extract [1,4,6,7]. However, due to insolubility the pyridine-extracted alum-precipitated extracts cannot be standardized in terms of allergenicity by QIE, and production batches are commercially controlled by direct RAST. This study confirms that the *in-vitro* (RAST estimated) potency is comparable to the *in-vivo* allergenicity evaluated by titrated intradermal skin test (IT).

Subjects and methods

Patients

Thirty-nine adult grass-pollen rhinitis patients selected to participate in an immunotherapy study were investigated. The patients had a positive history, skin test, and nasal challenge to grass pollen and had not previously been treated with grass pollen extracts. The patients did not receive any medical treatment known to interfere with skin testing 72-hr prior to the test. Informed consent was obtained and the study was approved by the local Ethics Committee.

Test extracts

Two batches of Allpyral® (Dome/Hollister-Stier) 5-grass mix labelled 1000 PNU/ml and another two ten-fold dilutions (100 and 10 PNU/ml) of the same batches were used for testing. The RAST activity of the two batches differed with a factor five measured by direct RAST (performed by the production firm). The difference in RAST activity was due to a decline in potency of one of the batches caused by storage. Histamine HCl 0.1 mg/ml was used as positive reference. Extracts and histamine were stored at 2–8°C when not in use. The study was completed within 3 months.

Skin testing

Intradermal test (IT) was performed as previously published [8]. In short, double determinations of three ten-fold dilutions (10, 100 and 1000 PNU/ml) of the two batches and quadruplicate histamine tests were performed on both forearms by the same experienced tester. According to a 'micropapule' technique, using 26-gauge needles, a volume of approximately 0.02 ml was injected, resulting in a 3 mm papule [8]. The tests were spaced 4-cm apart and randomized to different sites. The weals were recorded after 15 min and the area calculated by planimetry [8]. Using linear regression the dose-response curves (log-log) were computed. Tests with a coefficient of variation (SD%) > 40% and/or a coefficient of correlation < 0.9 were discharged as not valid [9]. In order to test for unspecific skin reactions, an alum solution was used as negative control.

Calculation of relative potency

Two different approaches were applied to estimate the relative potency of the high and low RAST-activity extracts. The horizontal distance between the two dose-response curves expresses the difference in potency [10]. The relative potency of each extract was expressed as the allergen concentration eliciting a weal equal in size to the mean of the weal areas elicited by the two different batches at the midpoint of the dose-response curve, i.e. at the concentration of 100 PNU/ml (Fig. 1). Furthermore, the relative potency was calculated as the allergen concentration eliciting a weal equal in size to the histamine reaction, i.e. the histamine-equivalent reaction (HEC) [1,11].

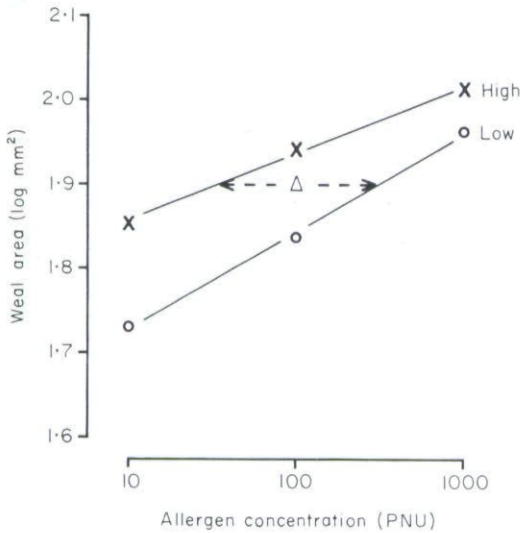


Fig. 1. Dose-response curves (log allergen concentration *vs* log weal area) of the high and low RAST-activity batches of Allpyral 5-grass mix. The distance between the two regression lines represents the difference in potency.

Statistics

Non-parametric testing (Wilcoxon's signed rank test) was used for comparison of relative potency and the slope of the dose-response curves. The dose-response curves were calculated by the least square linear regression method. The reproducibility of skin tests were evaluated as coefficient of variation ($\text{s.d.\%} = \text{s.d./mean weal area} \times 100$).

Results

Seventy-four per cent (29/39) of the test were accepted as valid ($\text{s.d.\%} < 40$, $r > 0.9$). Of the ten tests excluded from calculation, nine had a coefficient of correlation < 0.9 and in one case the exact location of high and low RAST-activity batches was uncertain. No unspecific reactions were elicited by the alum solution.

The median reproducibility of the weal areas was a $\text{s.d.\%}$ of 15% (25 and 75 quantile 14–22%). Parallelism between dose-response curves of the high and low RAST-activity batches in individual patients was found in only one-third of the tests. Parallelism between the slope of the dose-response curves of the two extracts was accepted in tests with a difference less than 0.030. The difference in slope between the two extracts, however, had no uniform trend. The median slope of the high RAST-activity extract was 0.110 (25 and 75 quantile 0.076 and 0.141). The corresponding figure for the low RAST-activity extract was 0.120 (0.090–0.170).

Figure 2 shows the difference in allergenicity estimated by intracutaneous test of both high and low RAST-activity extract using either the horizontal distance or the histamine equivalent reaction. Estimated by the horizontal distance, the median allergen concentration eliciting a weal of the same size as the mean weal of concentration 100 PNU/ml was 35 PNU (25 and 75 quantile 13–150 PNU) for the high RAST-activity extract. The corresponding figure for the low RAST-activity extract

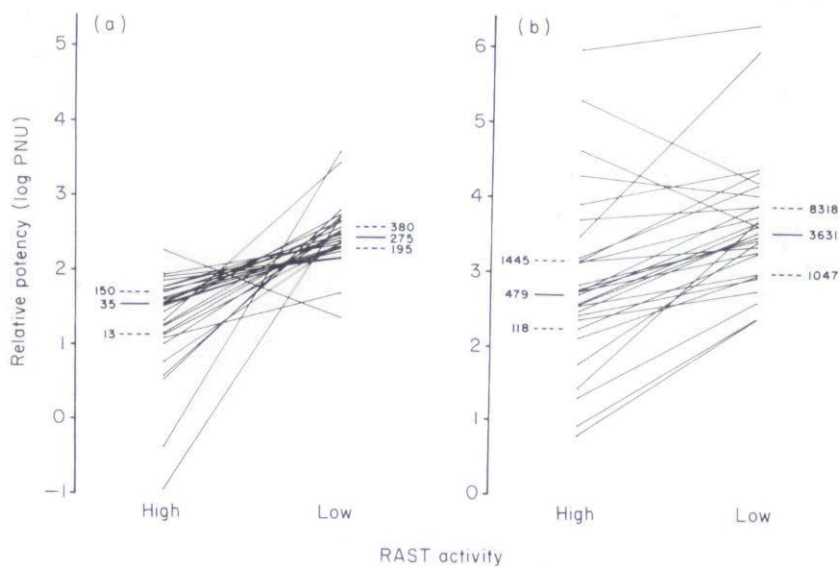


Fig. 2. The relative potency (expressed as allergen concentration) of the high and low RAST-activity batches estimated by (a) horizontal distance of the respective dose-response curves, and (b) histamine HCl 0.1 mg/ml equivalent reaction. Median and 25 and 75 quantiles are indicated.

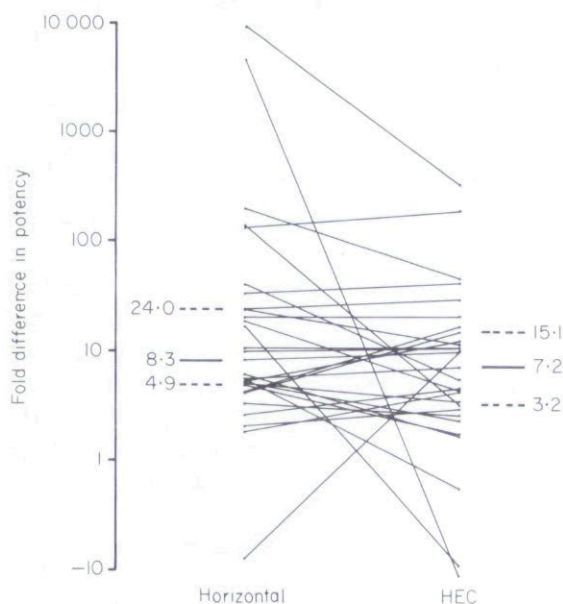


Fig. 3. The difference in potency (fold) between the high and low RAST-activity batch estimated by the horizontal distance and the histamine-equivalent reaction. Median and 25 and 75 quantiles are indicated.

was median 275 PNU (195–380 PNU) (Fig. 2a). As for the high RAST-activity extract the HEC-estimated skin reactivity was median 479 PNU (118–1445 PNU) and for the low RAST-activity extract median 3631 PNU (1047–8318 PNU). The median values for difference in potency between the two estimates were almost similar but with some individual differences (Fig. 3). Estimated by the horizontal distance the difference in potency was a median factor of 8.3 (25 and 75 quantile 4.9–24.0); the figure for the HEC-estimate was a median factor of 7.2 (3.2–15.1).

Discussion

The present data are the first to show that biological standardization of alum-precipitated extracts is possible by intracutaneous testing; secondly that a fairly good agreement exists between the *in-vivo* (IT) and *in-vitro* (RAST) estimated allergenicity.

The RAST activity of alum-precipitated extracts declines during storage and according to the present study this is due to a decreased IgE reactivity of the allergens, which might be explained by degradation of epitopes. This phenomenon is clinically implicated in immunotherapy. When changing to a new batch, a considerably higher dose of IgE-reactive allergen might be injected than indicated from the labelling and, thus, a risk of exceeding the threshold of the patient is introduced. As systemic side-effects in immunotherapy are primarily IgE mediated, knowledge of the IgE reactivity of a therapeutic extract is essential for the safety of the treatment. The biological potency of alum-precipitated therapeutic allergen extract can easily be estimated by quantitative skin test as well as by RAST. The agreement between the RAST- and intracutaneously-estimated allergenicity (a factor 5.0 *vs* 7.2/8.3, respectively) is quite acceptable considering the biological variation of RAST as well as skin test.

The RAST and skin test are estimates of the total activity and not of the qualitative composition of allergenic determinants [1,12,13]. A qualitative difference between the two allergen preparations examined, therefore, might explain the non-parallel dose-response curves. The alum precipitation in the manufacturing of extracts, however, prevents qualitative control of the epitopes by means of quantitative immunoelectrophoresis [1,4,6]. Qualitative control by the use of monoclonal antibodies, as described by Baldo [12], might be a practical solution to the problem.

The biological potency was evaluated by two different estimates: the horizontal distance and the histamine-equivalent reaction. In spite of a close and non-significant difference between the two estimates, the former is to be preferred as the histamine-equivalent reaction in some patients was based on extrapolation of the dose-response curve and, thus, not as exactly determined as the horizontal distance. Furthermore, this approach renders the possibility of labelling the therapeutic extracts in international units by simultaneous testing with an international standard [2,3,10]. An unsolved problem, however, is that the biological standardization of therapeutic extracts is still only in relation to the IgE reactivity, which *per se* might not be of importance in immunotherapy. As knowledge about the exact immunological mechanisms underlying immunotherapy is insufficient, standardization in relation to an immunological reaction cannot be accomplished now but might be possible in the future.

The present study has shown that the biological activity of alum-precipitated grass pollen extracts as estimated by RAST is in close agreement with the skin-test estimated activity. Because of the simplicity of performing an *in-vitro* test with standardized sera, this method is to be preferred for routine control of new production batches rather

than the time-consuming skin-test titration on larger groups of allergics. The data are based on testing two batches of grass pollen extract alone and before being generally accepted they need to be extended to other allergen systems.

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