

Challenge tests and specific IgE in hay fever sufferers

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Summary

The relationship between challenge tests, specific IgE and seasonal effects in hay fever sufferers was studied. There were significant relationships between nasal challenge, conjunctival challenge and skin-prick test threshold doses, although the skin and conjunctiva tended to be less sensitive than the nose. A seasonal effect was apparent on nasal challenge, but not conjunctival challenge or skin-prick testing, with lower threshold doses recorded after the pollen season than in midwinter. There was a significant relationship between specific IgE and nasal challenge, but not conjunctival or skin-prick test threshold doses.

Introduction

Challenge tests have been used in the diagnosis of allergy to airborne allergens and to assess the outcome of various therapeutic manoeuvres. The diagnosis of hay fever is usually made on the basis of a history and a positive skin-prick test while the outcome of immunotherapy may be assessed using nasal or conjunctival challenge tests. Nasal challenge is a time-consuming procedure, fraught with technical difficulties; conjunctival challenge is simple and repeatable with a clear end point, but can be uncomfortable; skin-prick testing is simple, repeatable and very acceptable to patients.

While hay fever is characterized by detectable levels of pollen-specific IgE, it is not clear to what extent end organ sensitivity is dependent on absolute levels of circulating specific IgE. A positive RAST is highly likely to predict a positive challenge test in patients with allergic asthma or rhinoconjunctivitis [1], and symptom scores have been shown to correlate well with specific IgE levels in patients with severe ragweed allergy [2,3]. Schumacher, however, was unable to show a relationship between specific IgE and nasal sensitivity [4].

This study attempted to establish whether there is a relationship between the nasal challenge, the conjunctival challenge and the skin-prick test, to observe the effect of season on the results of these tests and to assess the influence of specific IgE levels on end organ sensitivity.

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Patients and methods

Thirty patients [mean age 22.3 years (range 13–45)], with hay fever were studied, fifteen were female. All patients were skin-prick test positive to Timothy grass pollen and twenty-seven were also positive to at least one other common airborne allergen. Seventeen patients had mild intermittent symptoms of perennial rhinitis, not requiring regular treatment. All patients had detectable levels of IgE specific for Timothy grass pollen (SIgE). SIgE was measured by the RAST technique (Pharmacia, Uppsala, Sweden) and expressed as Phadebas RAST units/ml (PRU/ml), where a RAST grade 0 is equivalent to 0.35 PRU/ml and RAST grade 4 IV is equivalent to 17.5 PRU/ml.

Nasal and conjunctival challenges were performed with purified Timothy grass pollen antigens (Pharmacia) dissolved in normal saline and discarded after 7 days. Concentrations of 10^3 , 10^4 and 10^5 biological units/ml (BU/ml) were used for the nasal and conjunctival challenge and the skin-prick test.

Nasal airway resistance was measured using passive anterior rhinometry (NART P. K. Morgan, Chatham). The resting nasal airway resistance was first established. This was to ensure that the nose was not excessively blocked and that the resting resistance was stable and had not changed appreciably since the last challenge test. The mean of five measurements taken over 1 min was recorded and the measurements repeated at 5-min intervals for 20 min. The resting baseline resistance was the mean of the four sets of measurements. The nostril with the lowest resting resistance was used for the subsequent challenge and the same nostril was used throughout the study period. Two hundred microlitres of saline diluent were applied to the nostril via a coarse nasal spray. A further four sets of measurements were taken, as above, at 5-min intervals for 20 min, starting 5 min after the saline challenge. The mean of these readings was called the baseline resistance. Two hundred microlitres of pollen antigen in saline, in increasing concentrations from 10^3 to 10^5 BU/ml, were applied to the nostril at 20-min intervals, with serial recordings made at 5-min intervals as after the saline challenge. The aim of the test was to find the dose of pollen that gave a three-fold increment in nasal airway resistance compared with baseline resistance, in the mean of at least one series of measurements. When this was achieved, the test was completed and the concentration of pollen causing the increase was called the threshold dose. Because of the small number of patients sensitive to 10^5 BU/ml, these patients were grouped with patients sensitive to 10^4 BU/ml for statistical analysis. This protocol has proved to be acceptably repeatable, with the same threshold dose required for eight out of ten paired challenge tests performed 1 week apart.

For conjunctival challenge, one drop of normal saline followed by increasing concentrations of pollen antigen was applied to alternate eyes at 10-min intervals. The concentration of pollen antigen causing redness and itching of the conjunctiva was called the threshold dose. Four subjects failed to respond to the maximum concentration of pollen, 10^5 BU/ml, but are grouped with patients sensitive to 10^5 BU/ml for statistical analysis. The same threshold dose was required in nine out of ten paired conjunctival challenge tests performed 1 week apart. The skin-prick test was performed according to the technique described by Pepys [5]. The same pollen concentrations were used as in the challenge tests, dissolved in a glycerol phenol and albumin solution, together with a diluent control and 1 mg/ml histamine reference solution in normal saline, prepared fresh each week. The concentration of pollen giving a weal equal to, or greater than, the histamine-induced weal was called the threshold dose. The coefficient of variation of weal size for skin-prick testing was 11%.

Tests were performed between August 1983 and September 1983 and January 1984 and February 1984.

Continuous data were analysed using parametric statistics, Student's paired and unpaired *t*-test (2-tailed) and one-way analysis of variance. Non-continuous interval data was analysed using the non-parametric Kendal's Rank Correlation Test.

Results

Seasonal effects on challenge tests

Nasal challenge. Twenty-three subjects underwent nasal challenge after the 1983 pollen season and again in midwinter 1984. There was a significant fall in sensitivity over the winter with nine subjects requiring a 1 log increment and three subjects a 2 log increment in threshold dose. None showed an increase in sensitivity ($P < 0.001$) (Student's paired *t*-test).

Conjunctival challenge. Thirteen subjects had conjunctival challenge tests performed post pollen season and midwinter. Three (23%) required a 1 log increase and one a 1 log decrease in threshold dose in winter ($P > 0.05$). Twelve of the thirteen subjects underwent nasal challenges and of these eight (67%) required a 1 log increase in threshold dose in the winter. The group may be too small for meaningful analysis, but the trend towards a decrease in nasal sensitivity in the winter is not apparent on conjunctival challenge.

Skin-prick test. Twenty-seven subjects underwent skin-prick testing post pollen season and midwinter. No significant seasonal variation was noted, with four subjects requiring a 1 log increase and five a 1 log reduction in threshold dose in the winter.

Relationship between challenge tests. There was a significant correlation between threshold doses for nasal and conjunctival challenges ($\tau = 0.85$), nasal challenge and skin-prick testing ($\tau = 0.69$), and conjunctival challenge and skin-prick testing ($\tau = 0.72$) when measured midwinter (Kendal's Rank Correlation: $P < 0.001$) (Fig. 1).

Specific IgE

Seasonal variation. There was a significant reduction in SIgE in the winter. Post pollen season SIgE, mean $\pm$ s.d.: 92.9 ± 84 PRU/ml; midwinter: SIgE 52.4 ± 34 PRU/ml (Student's paired *t*-test: $P < 0.001$).

SIgE and nasal challenge. There was a significant relationship between nasal challenge threshold dose and SIgE, whether measured in post pollen season ($P < 0.01$) or midwinter ($P < 0.03$) (Student's unpaired *t*-test), with the most sensitive subjects reacting to 10^3 BU/ml having a higher mean SIgE than those reacting to 10^4 and 10^5 BU/ml (Fig. 2).

SIgE and conjunctival challenge. There was no significant relationship between SIgE and conjunctival challenge threshold dose whether measured post pollen season or midwinter (one-way ANOVA) (Fig. 3, winter values only).

SIgE and skin-prick testing. There was no significant relationship between SIgE and skin-prick threshold dose (Fig. 4, winter values only). There was a weak but significant relationship between weal size using an antigen solution of 10^4 BU/ml and log SIgE ($r = 0.39$; $P < 0.05$; $n = 30$) but not for 10^3 BU/ml ($r = 0.24$) or for 10^5 BU/ml ($r = 0.24$). There was no significant correlation using any solution when measured post pollen season.

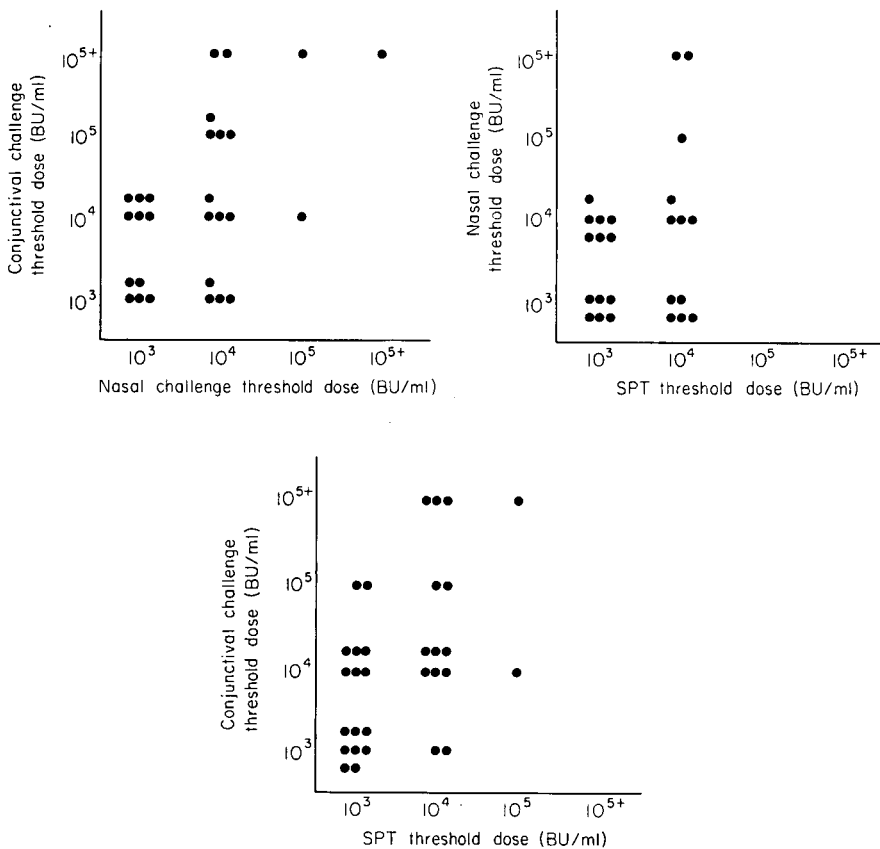


Fig. 1. Relationships between threshold doses for nasal challenges, (a) conjunctival challenges, (b) and (c) skin-prick tests performed in the winter. A threshold dose of 10^{5+} BU/ml indicates those subjects who do not react to the highest pollen concentration on conjunctival challenge.

Discussion

We have found a quantitative relationship between levels of SIgE and nasal sensitivity to grass pollen not previously described. Specific IgE levels have been related to symptom scores but only in patients with severe ragweed allergy [2,3]. Pepys was unable to find a correlation between RAST score and nasal threshold dose [5], although the challenge test in this study used only a subjective assessment of nasal blockage and the development of rhinorrhoea and sneezing as end points. Using a crude allergen extract of *Lolium perenne* and measuring SIgE using commercial RAST discs, Schumacher was unable to find a significant difference in SIgE levels between patients of differing nasal sensitivities [4]. The RAST discs in our study were specific for the antigen used and any discrepancy between the challenge antigen and the specificity of the measured SIgE may have accounted for the lack of relationship in Schumacher's patients. There was considerable overlap in SIgE levels in our study with some very low levels associated with high sensitivity and some high levels associated with low sensitivity. High sensitivity associated with low SIgE level is presumably the result of the nasal hyperactivity described in subjects with rhinitis [6]. It may be associated, to some extent, with a non-specific 'priming effect' [7] associated with perennial allergy, which many of the subjects had. SIgE levels were measured in batches throughout the

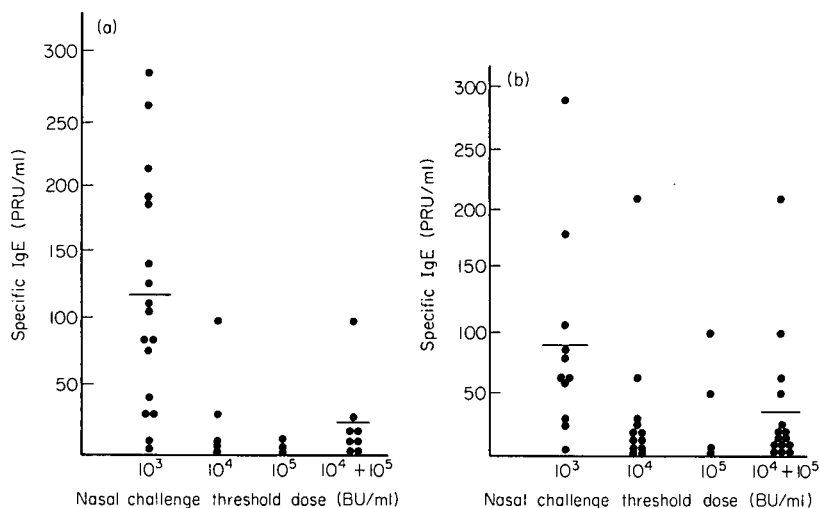


Fig. 2. Relationship between specific IgE and nasal challenge threshold doses (a) post pollen season and (b) winter values.

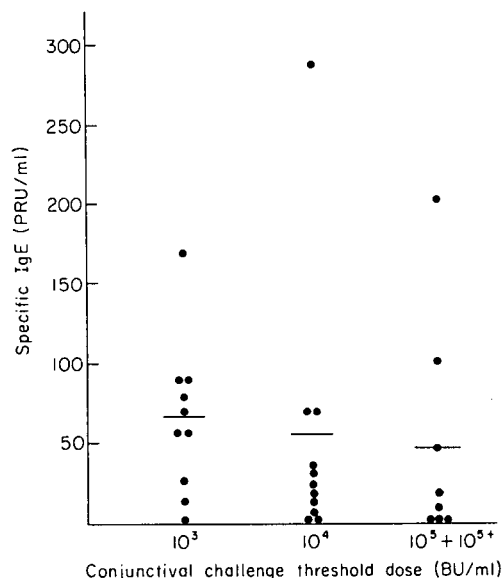


Fig. 3. Relationship between specific IgE and conjunctival challenge threshold doses, winter values.

year, the inter-assay variability being in the order of 10–20%. It is possible, therefore, that a closer relationship between SIgE and nasal sensitivity may have been evident if SIgE levels had been measured in one batch. So while SIgE levels are important in determining nasal sensitivity, reflecting as they do the number of IgE molecules on mast cells and basophils, which in turn will influence sensitivity to antigen [8,9], other factors must also be important. For example, although mast cell and basophil sensitivity to antigen triggering of mediator release is dependent on the number of IgE

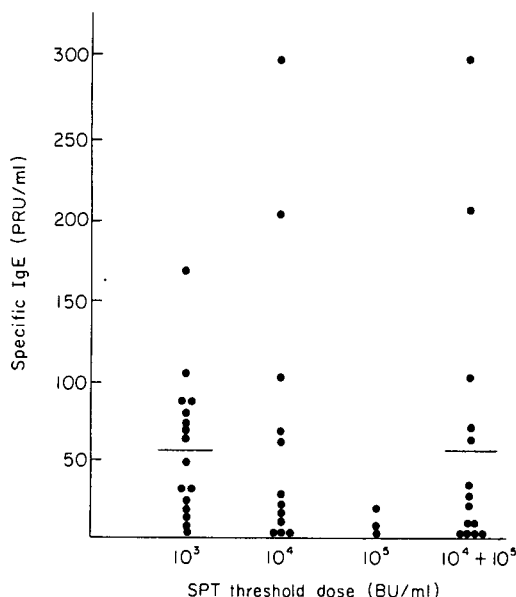


Fig. 4. Relationship between specific IgE and skin-prick test threshold doses, winter values.

molecules on the cell surface, intrinsic sensitivity does vary between subjects [10]. Nasal reactivity may vary because of differences in parasympathetic tone [11]. The number of mast cells and basophils present *in situ* may be an important factor, and finally, mediators and other local cellular mechanisms, which serve to regulate the immune response, may also be relevant.

In contrast to nasal sensitivity and in agreement with another study [12] no relationship was evident between conjunctival sensitivity and specific IgE levels. As regards the skin-prick test, Pepys found good agreement [5], about 85%, between a positive RAST and a positive skin-prick test and others have found a good correlation between specific IgE levels and threshold dose [13] and weal size [14–16]. This study found only a poor and erratic relationship between IgE level and weal size, with a correlation coefficient similar to that found by Perrin *et al.* [17]. Some of these discrepancies again may be due to the purity of the antigens used and the specificity of the IgE assay.

Nasal airway resistance can be influenced by a host of environmental factors and it is, perhaps, surprising that the relationship between SIgE levels and nasal sensitivity was so good, while the relationship with the much less reactive conjunctiva and skin was so poor. A possible explanation might be that the nasal mucosa, being so reactive, is presumably very sensitive to mast cell and basophil-mediator release. The extent of mediator release is dependent on the effector cell sensitivity to antigen, which in turn is related to serum IgE levels [8–9]. The allergic reaction in the conjunctiva and skin may be more closely under the influence of local homeostatic factors.

Despite the absence of a relationship between SIgE levels and conjunctival challenge and skin-prick test threshold doses, there remains a statistically significant correlation between the three challenge tests. There is, however, a tendency for the conjunctival and skin-prick threshold doses to be higher than the corresponding nasal challenge dose, emphasizing that the nose is generally the more reactive organ.

Repeated exposure to antigen increases nasal sensitivity in a non-specific manner, the effect lasting for several weeks. This has been referred to as the priming effect and is not related to levels of SIgE [7] but is likely to be due to IgE-mediated inflammation with local recruitment of mast cells, basophils and other inflammatory cells. Thus, nasal sensitivity tends to be greater at the end of the pollen season than midwinter. No such seasonal variation was found by Schumacher [4] but it is not known how high the pollen count had been around the time of the measurements. If the count had been low, there would have been little in the way of a priming effect and seasonal variation would not have been apparent. There was no consistent seasonal variation in skin-prick threshold dose and this might be expected since the epidermis is not a shock organ for pollen and there would be no increase in local IgE-mediated inflammation. The variation in SIgE levels would be unlikely to influence the skin-prick reaction since there is no relationship between the two. The lack of a definite trend towards increased conjunctival sensitivity in the summer may be due to the lack of a prolonged inflammatory reaction resulting in increased reactivity, reflecting a tighter homeostatic control than is apparent in the nose.

Finally, it has been suggested that a sneeze count and measurement of nasal secretions should be included in a nasal challenge protocol to achieve an acceptably repeatable test [18]. However, in this study neither of these parameters were considered helpful in improving the sensitivity or the repeatability of the nasal challenge test. In eighty-four consecutive nasal challenges, no sneezing was provoked by forty challenges, despite a three-fold or more increment in nasal resistance. Sneezing did occur in thirty-four challenges but in only twenty did subjects sneeze more than three times. Sneezing rarely occurred in the absence of a three-fold increment in nasal resistance and never exceeded more than two sneezes. Multiple sneezes only occurred with considerable nasal blockage, such that measurement of nasal resistance became unreliable. Since nasal resistance measurements were made every 5 min, collection of nasal secretions was not really practicable, secretions inevitably found their way on to the tracheostomy tube and subjects frequently involuntarily sniffed or blew their noses, despite instructions to the contrary. It was felt that collection of nasal secretions would be too inaccurate to be helpful.

To summarize, there is a significant relationship between nasal challenge, conjunctival challenge and skin-prick test threshold doses, suggesting that these tests may be giving equivalent information. In view of the relative difficulties associated with the nasal challenge tests, as well as the effects of seasonal exposure on threshold dose, the conjunctival challenge and skin-prick test may be more suitable tests for assessing the results of therapeutic interventions, such as immunotherapy. While there is a significant relationship between nasal sensitivity to pollen antigen and circulating SIgE, it is evident that other factors must also have a role in determining end organ sensitivity.

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