

5. Evaluation of changes in skin sensitivity by means of skin tests

The same principles as described under 4.1 are also valid for estimation of changes in skin sensitivity. Due to the log/log dose response relationship of allergens, the most common measure of change in skin sensitivity, change of weal size (1,16,21), is not valid. So called end point titration has a low precision and should not be used (18). Instead, methods based on parallel line bioassay (6,9,12), estimating the change in concentration needed to obtain the same weal response before and after treatment, should be preferred. Changes in skin reactivity given in change in concentration of allergen inducing a certain skin response, e.g. by parallel line assay, methods estimating the concentration eliciting a skin response corresponding to that of a reference substance such as codeine (23) or histamine (9), can easily be compared to e.g. changes in concentration of allergen inducing a positive challenge test in shock organs or changes in antibody titers. All these measures of sensitization show a normal distribution after logarithmic transformation (5,7,8,9).

There are a few reports showing decreased skin reactivity as measured by the size of the LPR (4,12,18).

5.1 SKIN PRICK TEST (SPT) METHODS FOR ESTIMATION OF CHANGE IN SKIN SENSITIVITY

5.1.1. *Methods for estimation of change of early phase reaction (EPR)*

5.1.1.1. WEAL SIZE

Most authors evaluating the change in skin sensitivity induced by immunotherapy or pharmaceu-

tical treatment have used the change in weal size to illustrate changes in skin reactivity. Due to the log/log relationship between changes in skin prick test response and allergen concentration only very large differences in skin sensitivity will be detected when using a single fixed concentration of allergen. The decrease in absolute size (21) or in % of size (1, 16) after treatment compared to before treatment is therefore not recommended as the method of choice. Furthermore, a limited decrease in weal size indicates a marked decrease in skin sensitivity, e.g. a tenfold decrease in skin sensitivity means a reduction in weal diameter from 6.8 mm to 4.5 mm and a hundred-fold decrease corresponds to a decrease in weal diameter from 6.8 to 3 mm.

5.1.1.2. PARALLEL LINE BIOASSAY

In 1984, Dreborg proposed (6) the use of parallel line bioassay (12) for evaluation of changes in skin sensitivity. Since then there has been several reports using this method (1,2,9,11,15,17,18). Most of these have used the system of Dreborg (6), testing in at least duplicate, at least three ten-fold concentrations of freshly reconstituted allergen preparation from the same batch before and after (immuno)therapy. The principle is illustrated in Fig. 1 a. This method should be considered the reference method.

5.1.1.3. METHODS BASED ON MEDIAN SLOPE

From this relatively complicated method, Dreborg et al (9) derived two other less laborious methods based on the same principle as the simple methods for BS (See Chapter 4). Both are based on median slope (0.4 when the area and 0.2 when the mean diameter of allergen induced weals are used). This is correct, provided changes are inves-

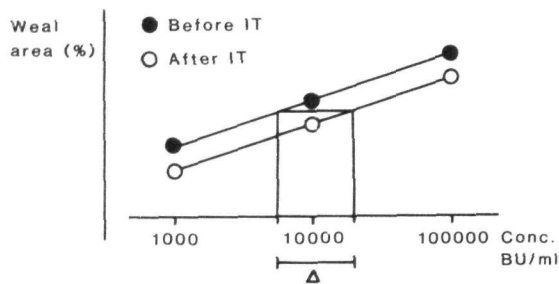


Figure 1 a. The principle of estimation in change in skin sensitivity by parallel line bioassay (4,8,11). Three concentrations, e.g. 1 000, 10 000 and 100 000 BU/ml of a freeze-dried allergen preparation are tested in at least duplicate before and after immunotherapy. The difference in concentration eliciting the same skin response is estimated by regression line analysis. Since skin sensitivity decreases during immunotherapy (or anti-allergic drug therapy) allergens should be 10–100 times stronger after than before therapy. No reference substance is needed.

tigated in samples of patients. The first method uses only one and the same concentration of allergen and histamine reference tested in duplicate before and after therapy. The change in allergen concentration needed to elicit a weal of the same size as a reference substance, e.g. histamine HCl 10 mg/ml (Fig. 1 b) is estimated. The other method is also based on the same principle. First, only one concentration of allergen is tested in at least duplicate. If the weals are of similar size as that of the reference then no further tests are performed, if smaller, then two ten- and hundred-fold higher concentrations of allergen are tested. Opposite,

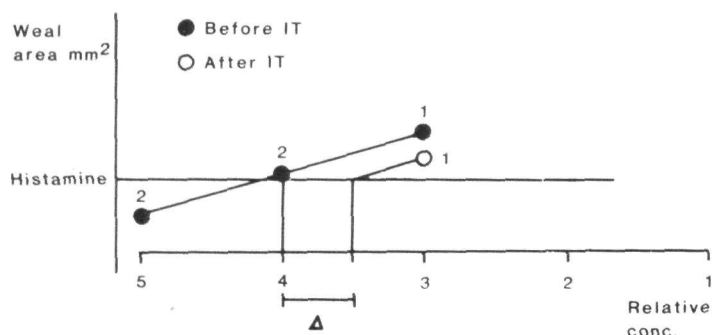


Figure 1 c. The simple method proposed by Dreborg et al (8). First one concentration (second dilution, third concentration) of allergen and the histamine reference are tested in at least duplicate. Provided the allergen weal has a size similar to that of the histamine reference, then no further tests are needed. If the response to allergen deviates much from that to histamine, then two ten and hundred-fold higher or lower concentrations of allergen are tested.

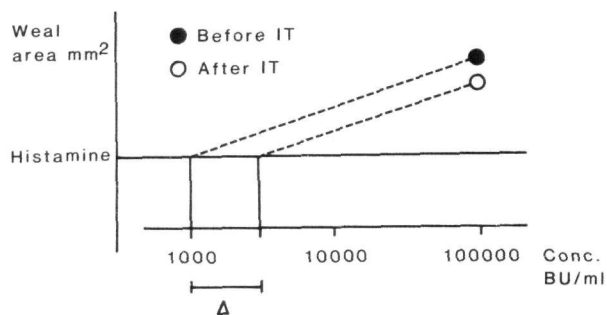


Figure 1 b. One and the same concentration of allergen and histamine 1 or 10 mg/ml is tested before and after therapy. Provided the allergen concentrations are high enough, results will be obtained from all patients. Since many extracts are weak, patients often turn negative, which makes evaluation difficult. The influence of the approximation of the allergen slope (0.4) can be pronounced, if most patients react with a weal much larger than that of histamine.

when the first concentration induces weals larger than those of the reference, then two ten- and hundred-fold lower concentrations of allergen are tested. Only results obtained with one of these concentrations, that inducing a weal most similar in size to that of histamine HCl 10 mg/ml are used for calculations (Fig. 1 c).

The drawback with the first method is that in some patients the concentration of allergen may be too high and especially after immunotherapy many patients will turn totally skin negative if the allergen concentration used is too low. The second method is a little more laborious, but has the advantage, that provided the concentration of allergen of the stock solution is high enough, changes in skin sensitivity can be estimated in all patients without any risk of inducing too large reactions.

5.1.1.4. ALLERGEN CONCENTRATION ELICITING A WEAL SIMILAR TO THAT OF A REFERENCE SUBSTANCE

The end point defined as the allergen concentration giving a weal size similar to that of the positive control, 9 % codeine phosphate, has been used as a measure of skin sensitivity in studies of allergen immunotherapy using house dust miet (23), grass pollen (24,25) and *Alternaria* (26) allergen extracts. Moreover, with this method a significant correlation was found between the end

point titer and the severity of the pollen season (25).

5.1.1.5. PRECISION OF THE ESTIMATE

The two simple methods correlate well to results obtained by parallel line bioassay in the same patient (9).

5.1.1.6. DOSE EFFECT (CHANGE IN SKIN SENSITIVITY) RELATIONSHIP

Using parallel line bioassay or the two simplified methods related above, the decrease in skin sensitivity during one year of immunotherapy varies between a few times and 300 times (8,15,17). In two studies, the effect of different top doses of allergen has been studied. Results fit roughly to a linear dose response relationship ($\log [\text{change in skin sensitivity}] = a + b \log [\text{top dose of allergens}]$) (12), but further studies are needed.

5.1.2. *Methods for estimation of change in late phase reaction (LPR) by SPT*

There are no methods in the literature for estimation of changes in LPR by SPT.

5.1.3. *Recommendations for estimation of change in skin sensitivity by SPT*

The Subcommittee recommends that changes in concentration of allergen is used as a measure of change in skin sensitivity and that parallel line bioassay is used as the standard method. Provided more than 10 patients are included in clinical trials the second method proposed by Dreborg (9) (with several allergen concentrations) is convenient. For practical purpose the first method of Dreborg et al (9) may be used in the routine with or without transformation of weal size to change in concentration inducing a weal of the same size as histamine HCl 10 mg/ml (9).

Alternatively the concentration of allergen inducing a weal response similar to that of a reference substance can be used (23).

5.2. INTRACUTANEOUS TEST METHODS FOR ESTIMATION OF CHANGE IN SKIN SENSITIVITY

5.2.1. *Methods for estimation of change in early phase reaction (EPR) by ICT*

When ICT has been used for estimation of the decrease in skin sensitivity, then weal size or ICT end point has been the most common parameters studied. However, the method employed by Turkeltaub et al for estimation of the relative potency of allergenic preparations in relation to a standard (23) can be adopted for this purpose. The same freeze-dried allergen preparation should be used before and after immunotherapy (or other form of therapy). The change in allergen concentration for induction of Σ 50 mm of erythema diameters should be estimated. As mentioned in chapter 4, the slope of the erythema dose response relationship is steep. This method might be the most precise method, suitable for estimation of changes in skin sensitivity in small samples of patients, but further studies are needed.

5.2.2. *Methods for estimation of change in late phase reaction (LPR) by ICT*

5.2.2.1. METHODS

Pienkowski et al (20) and Fling et al (13) have described methods. The concentration recommended for ICT (20) was 10 times the concentration eliciting a 4 + reaction according to their system. Both groups draw the contours of the swelling at 4 and 8 hrs (20) and 6 hrs (13), respectively. Then, the drawings were transferred to a record sheet with a translucent tape. The diameters were measured with a ruler (20) and the area was estimated by a digitizer (13), respectively. The contours of the LPR swelling is in most cases vague. Therefore, it is not possible to refer to any well defined method.

5.2.2.2. DOSE RESPONSE RELATIONSHIP

The difference in size of the LPR between patients treated with a potent ragweed extract and un-

treated patients was significant ($p < 0.001$, 20 and 67 mm in diameter, respectively, at 8 hrs) in the trial of Pienkowski et al (20). In the trial of Fling et al (13), comparing a mountain cedar extract to placebo, the difference between decrease in LPR was also significant ($p < 0.005$). Furthermore, Dieges et al (4) found that the late phase reaction totally disappeared in patients given grass pollen IT.

5.2.3 Recommendations for estimation of change in skin sensitivity by ICT

The Subcommittee recommends that the method of Turkeltaub et al proposed for estimation of differences in potency between allergen preparations is adopted also for estimation of changes in skin reactivity. Detailed methods for estimation of changes in LPR must be further evaluated.

5.3 RECOMMENDATIONS FOR ESTIMATION OF CHANGES IN SKIN SENSITIVITY DURING THERAPY

The Subcommittee recommends that the methods described under SPT methods are employed for estimation of changes in skin sensitivity, either spontaneous changes or changes induced by drug therapy or immunotherapy. Further methodological studies are needed before ICT methods are generally employed for this purpose. Estimation of changes of LPR are probably of great importance. However, before any recommendations are made, the dose response relationship must be studied and methods for estimation of change in LPR must be validated.

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