

Original article

Basophil allergen threshold sensitivity: a useful approach to anti-IgE treatment efficacy evaluation

Background: Monitoring of the allergen sensitivity of a patient is most important for optimal patient care and a basic prerequisite for immunomodulating treatment. The objective of this study was to investigate how basophil allergen sensitivity can be applied in the monitoring of anti-immunoglobulin E (IgE) treatment.

Methods: Basophils from timothy grass pollen allergic patients were, by flow cytometry, analysed for allergen threshold sensitivity (CD-sens) by measuring CD63 up-regulation on CD203c-identified basophils. The results were compared with maximal percentage CD63 up-regulation at one allergen dose (CD-max), skin prick test end-point allergen titration, (SPT-sens), nasal provocation titration tests (nasal provocation titre) and serum IgE and IgE antibody concentrations.

Results: There was a significant correlation ($r = 0.50$, $P = 0.01$) between CD-sens and SPT-sens, CD-sens and the IgE antibody concentration in percentage of 'total IgE' (relative IgE antibody concentration) ($r = 0.72$, $P < 0.001$) as well as between CD-sens and nasal provocation titre ($r = 0.54$, $P < 0.05$) but, in contrast, CD-max did not correlate with any of the sensitization parameters, i.e. SPT-sens, nasal provocation titre, absolute and relative IgE antibody concentration or CD-sens. CD-sens could be used to monitor omalizumab treatment efficacy while, based on CD-max, four of seven symptom-free patients on omalizumab would have been classified as having ongoing allergy.

Conclusions: CD-sens seems to be very useful for the determination of a patient's allergen sensitivity and should be evaluated for the measurement and monitoring of anti-IgE treatment efficacy. CD-max, the conventional approach to basophil allergen challenge, which mirrors cell reactivity, gives incorrect information.

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Methods available for monitoring treatment of allergic patients consist of skin testing, target organ challenge tests and *in vitro* analyses of immunological and inflammatory factors. However, the evaluation of a patient on immunoglobulin (Ig) E-specific immunomodulation treatment (ESIT) needs a different approach, as many of the links of the allergy inflammation chain reaction are not available for analyses. Skin prick test (SPT) is simple and safe but not useful for quantification of sensitivity because the precision is very low (1). Provocation challenge tests can be used but are difficult to handle, time-consuming, not very quantitative and potentially dangerous for the patient.

Serological tests for IgE sensitization (2) are routinely used for allergy diagnosis and research, and can be used

to evaluate the degree of a patient's allergen sensitivity (3). However, although it is possible to quantify IgE and IgE antibody in serum of patients on omalizumab, the clinical significance is not clear and the approach to measure 'free IgE' is technically difficult and is unlikely to be used routinely.

Allergen challenge of basophils is an attractive *in vitro* surrogate for *in vivo* provocation. The approach based on flow cytometric detection of CD63 on basophils is useful (4) and the accessibility of the basophil-specific marker CD203c (5) has made it possible to follow basophil activation also in patients on ESIT.

The aim of this study was to investigate how basophil allergen sensitivity could be applied in the monitoring of anti-IgE treatment.

Material and methods

Subjects

Blood for cell analysis was collected in heparinized tubes from 27 patients with a clinical history of asthma and/or rhinoconjunctivitis and positive SPT and IgE antibody to timothy grass pollen. The study was performed outside the grass pollen season and all patients were drug free and had not previously been treated with allergen-specific immunotherapy.

Seven essentially symptom free, timothy allergic patients who had received ESIT (omalizumab; Novartis, Basel, Switzerland) for 4 years as part of a prospective, clinical trial (CIGE 0250011), were analysed for basophil allergen sensitivity and reactivity. In addition to omalizumab, the patients were taking inhalation, but not systemic, corticosteroids and some other asthma drugs, but no antihistamines during the days prior to testing. Four other patients received 3 months treatment with omalizumab and were followed before and once every month after ESIT.

All patients gave their informed consent to participate in the study, which was approved by the Ethics Committee of the Karolinska University Hospital, University of Linköping and the University of Gothenburg, Sweden.

Nasal provocation

Fourteen patients, referred to the ENT clinic at Malmö University Hospital, were subject to nasal provocation titration with a timothy allergen extract as part of the diagnostic work-up. The lowest allergen concentration eliciting symptoms in the eyes or airways, the nasal provocation titre, was used as a measure of nasal allergen sensitivity.

Definition of SPT-sens, CD-sens and CD-max

Skin prick test allergen titration was performed as previously described (4). For comparison of patients, the timothy allergen threshold dose corresponding to the wheal size of the histamine control was calculated, inverted, multiplied by 10 000, and termed SPT sensitivity, 'SPT-sens'. The higher the SPT-sens, the higher is the skin allergen sensitivity.

Basophil allergen sensitivity was analysed by flow cytometry after incubation with serial dilutions of timothy allergen (4). The term CD sensitivity, 'CD-sens' (4), was defined as the inverted value for the allergen concentration giving a 50% of maximum CD63% up-regulation multiplied by 100, and used to describe a patient's allergen-specific sensitivity. The higher the CD-sens, the higher the basophil allergen sensitivity. The intra-assay coefficient of variation (CV) was 13.1% and the interassay CV 5.4% (4).

The term CD maximum, 'CD-max', defined as the maximal percentage of CD63-positive basophils at one allergen concentration (300 SQU/ml), was determined following the recommendations given in a commercial kit (FLOW CAST; Bühlmann Laboratories, Allschwil, Switzerland). The allergen concentration of the timothy extract provided in the kit, as determined by IgE antibody inhibition was about 300 SQU/ml (BAG-G6 Lot 2423; Bühlmann Laboratories) when compared with the timothy pollen allergen extract used (ALK Timothy 100 000 SQU/ml, Batch 0000052706; ALK-Abello A/S, Hørsholm, Denmark).

IgE and IgE antibodies

The concentration of IgE (kU/l) in serum was determined by ImmunoCAPTM Total IgE (Pharmacia Diagnostics AB, Uppsala,

Sweden) and of IgE antibodies (kU_A/l) to timothy grass pollen allergen (g6) by ImmunoCAPTM Specific IgE (Pharmacia Diagnostics AB) according to the manufacturer's instruction. The relative IgE antibody concentration was expressed as absolute value in percentage of 'total IgE'.

Statistical analysis

Results are presented as geometric means [95% confidence intervals (CI)]. Correlation coefficients are calculated using Spearman rank correlation coefficient. $P < 0.05$ was considered as significant.

Results

Allergic patients

The SPT dose-response curves from 27 timothy allergic patients were parallel and the variation (in mm² of wheal reaction between duplicates) was high (mean CV 46%), corresponding to a SPT end-point allergen dose mean CV of 366%. The patient's SPT-sens geometric mean was 3.6 (4.6–78.5), CD-sens 16.4 (1.0–325) and CD-max 43.0 (12–100). Patients with a similar CD-sens could have very different CD-max (Fig. 1).

There was a significant correlation ($r = 0.50$, $P < 0.01$) between SPT-sens and CD-sens but not between SPT-sens and CD-max ($r = 0.30$) or between CD-sens and CD-max ($r = 0.32$). No significant correlation ($r = 0.30$) was found between CD-sens and absolute IgE antibody concentration (Fig. 2A) but with the relative IgE antibody concentration ($r = 0.72$, $P < 0.001$) (Fig. 2B). No significant correlation was found

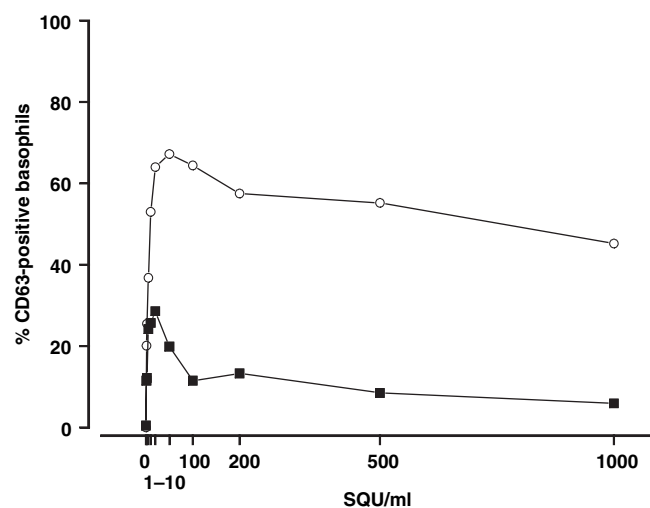


Figure 1. Two different patterns of dose-response curves after allergen stimulation. The two patients had almost identical CD-sens (○—○, CD-sens 34; ■—■, CD-sens 49) while there is a significant difference in CD-max. The patient with CD-sens 49 is very close to a negative CD-max, CD-max 16, at the commercially suggested allergen dose 300 SQU/ml.

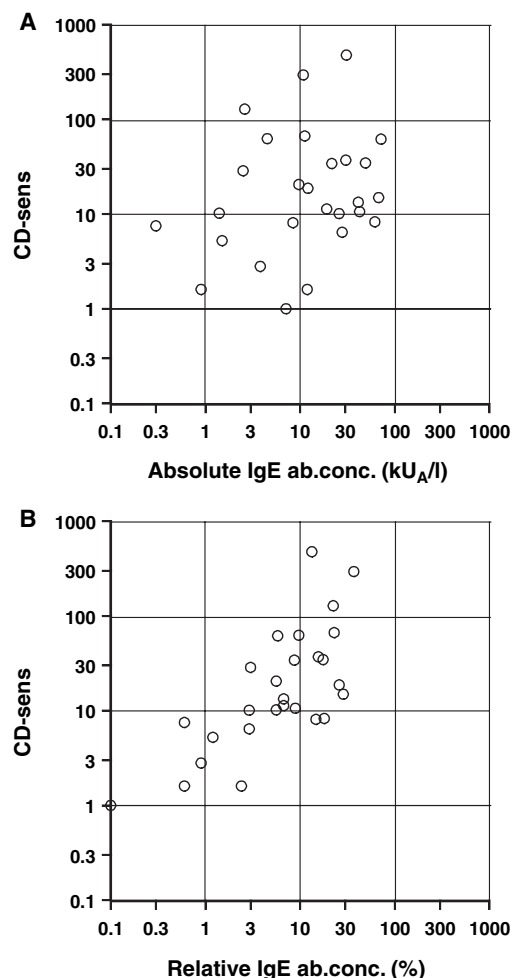


Figure 2. Absence of a correlation between CD-sens and the absolute IgE antibody concentration ($r = 0.27$) (A) contrary to a highly significant correlation with the relative IgE antibody concentration ($r = 0.72$; $P < 0.001$) (B).

between the absolute IgE antibody concentration and SPT-sens ($r = 0.41$) or CD-max ($r = 0.41$), or between the relative IgE antibody concentration and SPT-sens ($r = 0.34$) or CD-max ($r = 0.18$).

In 14 timothy allergic patients, there was a significant correlation between nasal provocation titre and CD-sens ($r = 0.54$, $P < 0.05$) but not with either CD-max, or absolute or relative IgE antibody concentration.

Anti-IgE treatment

Basophils from patients on ESIT compared with non-treated, allergic patients, had a significantly ($P = 0.002$) lower CD-sens, while their CD-max was barely significantly lower ($P < 0.05$) (Fig. 3). Of the essentially symptom-free patients on omalizumab all had a CD63% above 7% and a majority, four of seven, had a CD-max value within the 95% CI of the allergic patients. In contrast, only one of the ESIT patients had a weakly positive CD-sens.

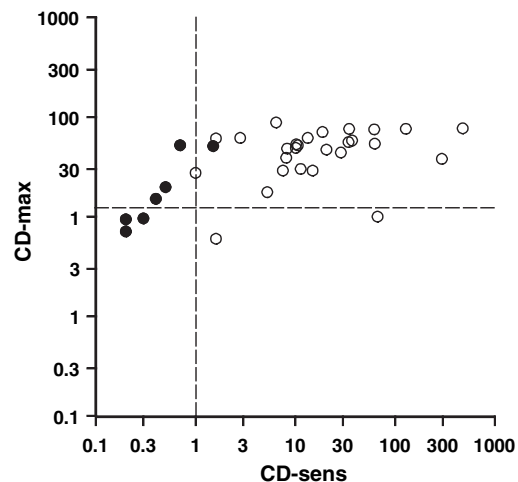


Figure 3. CD-sens and CD-max values for 27 allergic patients (○) and seven patients on omalizumab treatment (●). The dotted lines represent the lower level of the 95% CI for allergic individuals. All patients but one in the omalizumab group had a CD-sens below the 95% CI for allergic patients. In contrast, four of seven patients on omalizumab had a CD-max within the 95% CI, two of them actually above the mean of allergic patients.

Four other patients were followed before and after a short period of ESIT. The CD-sens dropped during the treatment and stayed low for 3–4 months (Fig. 4A), while CD-max increased markedly in two patients as the other two decreased moderately (Fig. 4B).

Discussion

The humanized monoclonal anti-IgE antibody omalizumab decreases levels of circulating IgE by binding to the constant region (Cε3) of the IgE molecule, thereby preventing it from binding to IgE FcεRI and FcεRII receptors (6). Omalizumab has been shown to reduce the level of free IgE by 96% within 3 days followed by a 73% decrease in FcεRI expression on basophils after 7 days (7), and over time nasal and SPT responses are significantly reduced (8, 9). These results are most promising, and now when Xolair is available for prescription there is an urgent need for a simple test to monitor treatment.

The general approach to identify the allergen(s) responsible for the symptoms is not useful for the evaluation of ESIT efficacy. For example, although the SPT is easy to perform the precision is not good enough to allow quantitative evaluation (1) and target organ provocations, like allergen challenge, is too complicated for routine use. An alternative is *in vitro* challenge of tissue sensitized *in vivo*. Basophil as target was extensively explored (10) before IgE was discovered (11). It was found that histamine release after challenge with one high allergen dose gave a response dependent on cell reactivity

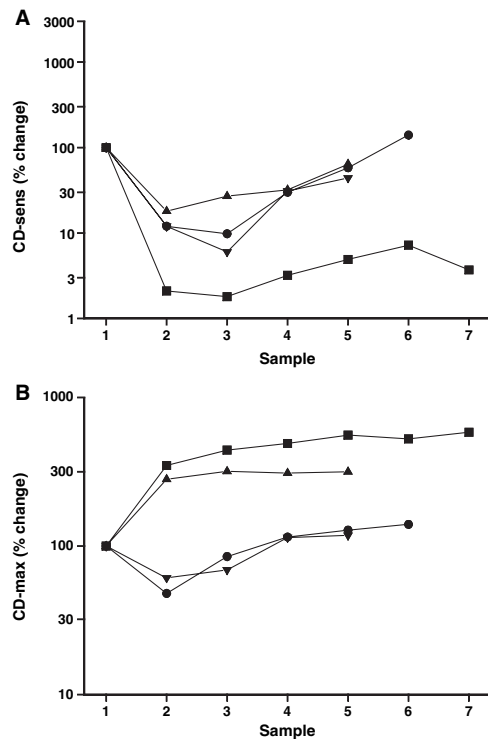


Figure 4. (A) Percentage change in CD-sens and (B) CD-max values in four patients on omalizumab treatment. Sample 1 was taken before the start of omalizumab, sample 2 after 3 months of treatment, and samples 3–7 were taken once a month after the treatment had been stopped.

while allergen threshold exposure was a measure of allergen sensitivity. In the early 1990s, it was shown that flow cytometric measurements of cell surface-exposed CD63 on basophils could substitute histamine assays (12). This principle has been commercially explored for allergy diagnosis. However, it is then recommended that basophils be challenged with one or a few high allergen doses, and a positive test is defined as a minimum, significant, percentage (usually 5–15%) of CD63-positive basophils (13). This one-dose allergen challenge approach is here referred to as CD-max. The clinical relevance of CD-sens and CD-max was evaluated by SPT-sens, nasal provocation titre and detection of absolute and relative IgE antibody concentrations.

In the present study, the basophils were challenged by serial dilutions of the allergen, and the basophil allergen

sensitivity, termed CD-sens, was calculated (4). A significant correlation was found between CD-sens and SPT-sens, nasal provocation titre and relative IgE antibody concentration. In contrast, no correlation was found between CD-max and any of these parameters. Thus, there is no relation between the degree of cell reactivity and allergen sensitivity. Whether measurement of cell reactivity gives any additional clinical information needs further study.

CD-sens seems to be useful in monitoring patients on ESIT. In almost symptom-free timothy allergic ESIT patients on long-term treatment, CD-sens drops to levels well below those observed in allergic patients, while all patients had a CD-max above what is often recommended as cut-off, i.e. 5% CD63%, four of seven patients even had a CD-max within the 95% CI of timothy allergic patients. Similar results (A. Nopp, unpublished data) were found for cat dander and house dust mite allergens. Thus, from CD-max data the clinician will get the impression that the patient is still allergic and inappropriate decisions, like withdrawal of anti-IgE treatment or addition of unnecessary drugs, might be taken.

Four patients who received omalizumab for 3 months had a decline in CD-sens during the treatment and stayed below the starting value for at least 3 months after the treatment. In contrast, in two patients CD-max increased 300–600% and they would falsely have been regarded as nonresponders of the treatment while their allergen sensitivity, CD-sens, in fact had decreased to 10–20% of the pretreatment values.

In conclusion, by challenging *in vivo* IgE-sensitized basophils with serial dilutions of the allergen, and determining the allergen threshold, CD-sens, a good quantitative measure of allergen sensitivity can be obtained. Commercial allergy tests based on CD63 up-regulation do not give any information on the degree of allergen sensitivity, which is most important when considering patients undergoing immunomodulating therapy, like anti-IgE.

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