

Short communication**Simultaneous exposure of several allergens has an additive effect on multisensitized basophils**

Background: Patients immunoglobulin (Ig)E-sensitized to more than one allergen in their environment often have more symptoms than mono-sensitized individuals, which indicates that the allergens may have an additive effect. In order to study if such an effect could be detected on the inflammatory, cellular level, multisensitized basophils were challenged with various dose combinations of two relevant allergens.

Methods: Basophils from patients IgE-sensitized to timothy/cat, birch/cat, timothy/mite and cat/mite were challenged with serial dilutions of different combinations of the two allergens. The basophil response was measured as CD63 expression analysed by flow cytometry.

Results: The doses of each allergen in the pair had an additive effect resulting in a shift of the dose–response curve to higher CD63 percentages and higher CD-sens.

Conclusions: If a patient has IgE antibodies and thus sensitized basophils to more than one allergen, to which he is simultaneously exposed, the additive effect should be considered. Even low concentrations of IgE antibodies could be of clinical relevance in such a situation.

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It is well-known that a patient allergic to cat and house dust mite, with symptoms to mites, gets worse, when exposed also to the cat. The most accepted explanation is a mucosal hyperreactivity because of the symptom-inducing inflammation (1, 2). However, it has also been suggested that the mast cells and basophils actually are more efficiently triggered by two, independent allergens.

Allergic inflammation is initiated by allergen molecules cross-bridging their corresponding, receptor-bound, immunoglobulin (Ig)E antibody molecules (3) on the mast cell or basophil cell surface. A total of approximately 1000 bridges are necessary to trigger the cell (4). It might be possible to accumulate the critical number of bridges by more than one, noncross-reactive allergen and its corresponding IgE antibody.

The aim of this pilot study was to perform allergen threshold challenge *in vitro* of basophils of patients IgE-sensitized to more than one allergen and to evaluate if the responses to each of two allergen-IgE antibody systems were accumulated. The clinical importance of such an additive effect is discussed.

Material and methods**Subjects**

Blood for cell analysis was collected in heparinized tubes from four subjects with a clinical history of asthma and/or rhinoconjunctivitis, a positive skin prick test (SPT) and IgE antibodies to

any two of the following allergens: timothy grass pollen, birch tree pollen, house dust mite and cat dander. All the individuals were drug-free and had not previously been treated with allergen-specific immunotherapy (ASIT). All the patients gave their informed consent for using their basophils in studies of allergic inflammation.

CD-sens

Basophil activation was detected by flow cytometry after incubation with serial dilutions of an allergen (ALK-Abello A/S, Hørsholm, Denmark; 5) or a mixture of two allergens and followed by staining for CD63 and CD203c. Basophil allergen threshold sensitivity, termed CD sensitivity, 'CD-sens' (5), was defined as the inverted value for the allergen concentration giving a 50% of maximum CD63% expression 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) for CD-sens was 13.1% and the interassay CV was 5.4% (6).

IgE and IgE antibodies

The serum concentrations of IgE (kU/l) were determined by ImmunoCAP™ Total IgE (Phadia AB, Uppsala, Sweden) and of IgE antibodies (kU_A/l) to timothy grass pollen allergen (g6), birch tree pollen (t3), *Dermatophagoides pteronyssius* (d1) and cat dander (e1) by ImmunoCAP™ Specific IgE (Phadia AB) according to the manufacturer's instructions.

Results

Basophils from a patient (AN) with IgE antibodies to timothy (3.9 kU_A/l) and house dust mite (7.4 kU_A/l) were challenged with decreasing concentrations, 200 to 1 SQU/l, of timothy allergen. The slope covered 200 to 5 SQU/l and the CD-sens was calculated to be 3.1. Similarly, the cells were challenged with mite allergen dilutions and a CD-sens of 0.2 was found. The test was repeated by mite allergen at either of three concentrations, 100, 200 and 500 SQU/l, which were added to each timothy allergen dilution before stimulating the basophils. A left shift of the challenge curve was seen (Fig. 1A); the more mite allergen that was added, the higher the CD-sens (8.0, 10.6 and 51.8 respectively) and the more to the left was the curve shifted.

Similar results were found with two other patients. One of them (KL; Fig. 1B) was sensitized to cat (9.3 kU_A/l) and mite (0.78 kU_A/l) and the other (IXN; Fig. 1C) to timothy (14.6 kU_A/l) and mite (9.5 kU_A/l). The CD-sens to timothy of patient IXN increased up to eightfold when simultaneously stimulated with mite 200, 500 or 1000 SQU/ml and that of patient KL to cat up to twofold when simultaneously stimulated with mite 50, 100 or 1000 SQU/ml. Basophils from patients sensitized to one allergen did not show any increase in CD63 response, when simultaneously stimulated with another allergen to which the patient was not sensitized.

A chess-board set up using basophils of patient KL and MLN showed that the increase in basophil CD63 expression was dose-dependent and that the two allergen-IgE antibody systems, cat/mite and cat/birch, had an additive effect (Table 1).

Discussion

Clinical studies indicate that patients allergic to and simultaneously exposed to several allergens have more symptoms than mono-sensitized individuals (1, 2). This could be because of an increased mucosal reactivity, which is well-known to develop during a period of symptoms. But it could also be the result of an additive effect of IgE antibodies, with different specificities, initiating the allergic inflammation behind the symptoms.

A mast cell or basophil is triggered to release its mediators, when a number of high affinity IgE receptors, FcεRI, are aggregated (3). This concentration in density of FcεRI receptors can be obtained by bridging of IgE antibodies to an allergen having at least two epitopes corresponding to the IgE antibodies (3). The critical number of bridged IgE molecules has been estimated to be in the order of 2000 out of a maximal number of some 500 000–1 000 000 IgE molecules on the cell surface (4).

Allergen threshold challenge of *in vivo* IgE-sensitized basophils is a most interesting surrogate for allergen challenge of an organ like the lungs in bronchial allergen inhalation or the skin in skin prick allergen titration. The lowest allergen dose, giving a significant basophil response, measured as cell surface CD63 expression, the so-called CD-sens, shows a significant correlation with

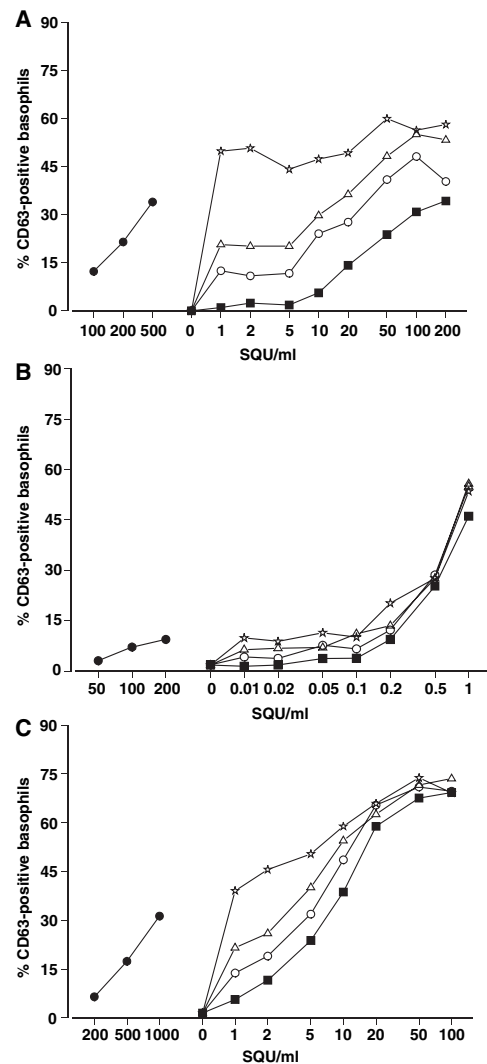


Figure 1. Basophils from three patients each immunoglobulin (IgE)-sensitized to two allergens were challenged with serial dilutions (■) of one of them: timothy 1–200 SQU/ml (A), cat 0.01–1 SQU/ml (B) or timothy 1–100 SQU/ml (C) mite allergen (●), to which the patients were also sensitized, was added, to each timothy or cat dilution, in increasing doses: 100 (○), 200 (△) and 500 (*) SQU/ml (A), 50 (○), 100 (△), 200 (*) SQU/ml (B), and 200 (○), 500 (△) and 1000 (*) SQU/ml (C). The basophils became further stimulated and the CD63 expression increased resulting in a shift of the dose–response curves to the left.

clinical allergen challenge tests (5). However, in contrast to allergen challenge of patients' *in vivo*, it is easy to handle, has a low CV and is independent of the clinical state of the patient. In addition to experimental situations (6), it seems to be a most promising approach to evaluate the efficacy of ASIT (A. Nopp, S.G.O. Johansson, L.O. Cardell, unpublished data).

When basophils sensitized with IgE antibodies to two, noncross-reactive allergens, e.g. timothy grass and *Dermatophagoides pteronyssinus*, are stimulated with increasing dilutions of one of the allergens, e.g. timothy, a dose–response curve with decreasing expression of

Table 1. Addition of increasing, from sub-threshold, doses of cat allergen extract to increasing doses of birch pollen allergen (patient MLN) or mite allergen (patient KL) extract results in an additive effect, indicated by a line at the border, on percent CD63 expression

		Birch SQU/ml					
		0	2	5	10	20	50
Cat SQU/ml	0	0	0	7.9	18.0	39.6	69.7
	1	1.4	2.8	10.8	19.2	42.6	71.0
	2	1.9	3.4	10.4	22.9	45.9	72.9
	3	4.5	6.0	12.3	19.4	49.0	75.0
	10	34.7	32.4	34.3	44.7	58.0	69.6
	20	42.0	38.5	42.5	51.7	60.8	79.3
		Mite SQU/ml					
		0	20	50	100	200	500
Cat SQU/ml	0	0	1.9	3.8	4.5	6.9	9.2
	0.02	0.2	0	0.05	3.3	5.6	8.2
	0.05	0.8	0	0.4	3.4	4.8	8.2
	0.1	1.5	0.4	1.5	4.4	8.4	13.1
	0.2	2.1	2.6	4.2	5.8	7.7	16.3
	0.5	2.5	7.1	10.9	13.1	17.8	20.5

CD63 is obtained. When, to each dilution of timothy allergen was added a low dose of the other allergen, *D. pteronyssinus*, in itself capable of giving only a weak stimulation, the timothy CD63 response curve is shifted towards increasing CD63 percentage and consequently higher CD-sens. Obviously, the CD63 expression of the two individual allergens is accumulated. Thus, the critical event is not how many bridges of IgE antibodies of one allergen specificity are created, but rather the number of bridges, independent of IgE antibody specificity.

There is a relation between the number of IgE molecules, some of which are IgE antibodies, in plasma and the number bound to basophil, and presumably mast cell, surface IgE receptors (4). Consequently, the lower the serum IgE antibody concentration, the more difficult it will be to trigger the basophils and the less symptoms of allergy will the patient experience, when exposed to the allergen in question. However, from this study it is obvious that IgE antibodies with different specificities can have an additive effect, i.e. if the basophils and mast cells are sensitized with small, even subthreshold numbers of IgE antibodies of different specificities they can 'join forces' and trigger the cells to release its mediator, if the patient is simultaneously

exposed to the corresponding allergens. And surprisingly, small amounts of allergen seem necessary.

The understanding of the clinical importance of the additive effect and its mechanisms should change our interpretation of the potential clinical importance of low serum IgE antibody levels. If a patient has a low serum IgE antibody concentration, e.g. to house dust mite, it would be judged as less clinically relevant. However, if the patient in addition has IgE antibodies to, e.g. cat dander, an allergen that he is simultaneously exposed to, the IgE sensitization should be seriously considered. Together, the two allergens can initiate an inflammation resulting in a clinically significant allergic disease. The clinical implication of such an additive effect has recently been observed in relation to wheezing in preschool children (2) where the strongest association of current wheeze was found for the sum of IgE antibodies to mite, cat and dog.

In order to estimate how common IgE sensitization might be to two or more noncross-reactive allergens, which could be present in the environment simultaneously, the prevalence was calculated among the serum samples sent to the Allergy Laboratory, Karolinska University Hospital, during 2004. A total of 6200 serum samples were analysed with two to four of the allergens house dust mite, cat dander, timothy grass pollen or birch tree pollen (total number of analyses: 19 800). The prevalence of IgE antibodies, in one serum sample, to either two of the allergens, varied between 9% and 20%, while 6% were positive to the combination of cat and mite plus either timothy or birch tree.

This study shows that IgE sensitization to an allergen should not be clinically evaluated on its own but in the possible context of multi-allergen sensitization and exposure. Depending on the environmental situation, 'packages' of likely concomitant allergens should be considered. In Sweden, the likely candidates could be cat and dog dander together with house dust mites as an 'indoor package', birch, grass and weed pollen as an 'outdoor, seasonal package' and cow's milk and egg white as a 'basic food package'.

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