

Short communication

Serum CD23 is not altered in gastroallergic anisakiasis, but correlates with the production of specific IgE and the amount of polyclonal stimulation

Background: Previous studies have shown elevated serum levels of the cytokines IL-4 and sCD23 in atopic patients and parasitic disease. Gastroallergic anisakiasis is an acute parasitic disease, accompanied by IgE-mediated clinical symptoms and an important increase of specific and total IgE.

Methods: Sixteen patients with acute urticaria/angioedema due to parasitism by *Anisakis simplex* after intake of raw or undercooked fish were selected, and serum samples were taken in the emergency room within 24 h (day 0; $n = 16$), after 1 month ($n = 16$), and after 6 months ($n = 10$). Serum samples were studied for specific IgE against *A. simplex*, total IgE, sCD23, and IL-4.

Results: Mean values for sCD23 did not change in the observation period. Only 4/16 serum samples showed measurable IL-4 levels. Specific IgE and total IgE levels were found to be elevated after 1 month; after 6 months, they fell to nearly basal values. There was a positive correlation between sCD23 and specific IgE at day 0 and follow-up ($r = 0.55$ – 0.69 , $P < 0.026$); a positive correlation between sCD23 and total IgE ($r = 0.54$ – 0.62 , $P < 0.056$). Basal sCD23 could moderately predict the percentual increment of total IgE in the first month ($r = 0.56$, $P < 0.038$).

Conclusions: Thus, it seems that interindividual variability of sCD23 is an important factor, with higher values predisposing to more production of unrelated IgE, independently of the parasite's action.

**A. Daschner¹, C. Cuéllar²,
A. Alonso-Gómez³, C.-Y. Pascual³,
M. Martín-Esteban³**

¹Sección de Alergia, Hospital Universitario La Princesa, Madrid; ²Departamento de Parasitología, Facultad de Farmacia, Universidad Complutense, Madrid; ³Laboratorio de Inmunoalergia, Hospital Universitario La Paz, Madrid, Spain

Key words: *Anisakis simplex*; sCD23; IL-4; polyclonal stimulation.

Dr A. Daschner
Sección de Alergia
Hospital Universitario La Princesa
C/ Diego de León 62
E-28006 Madrid
Spain

Accepted for publication 28 May 2001

IL-4 and sCD23 have been shown to be increased in atopy, but also in parasitic disease, IL-4 being the most important cytokine mediating IgE synthesis (1–4). CD23 and sCD23 have been implicated as positive or negative regulators of IgE synthesis, depending on the immune context (5, 6).

Gastroallergic anisakiasis is an acute parasitism, caused by the third-stage larva of *Anisakis simplex*, in which patients suffer from acute type-I hypersensitivity symptoms, indicating a new parasite contact with the host, as specific IgE antibodies, produced presumably in a previous acute parasitic contact, come into contact with their corresponding allergens and lead to a typical allergic response (7–9). New IgE antibodies are produced with each new contact with the live parasite, and a polyclonal expansion of IgE-bearing cells can be demonstrated by a close followup of specific and total IgE (10, 11).

Cleavage of surface CD23 leads to the release of sCD23, an effect which may lead to upregulation of IgE synthesis (1). In this respect, it is interesting to note that a major allergen of the house-dust mite, Der p 1, has

been shown to cleave proteolytically the low-affinity receptor for human IgE (CD23) (12, 13). The role of sCD23 in parasitic disease has not been fully elucidated, but it is tempting to assume that a similar mechanism involving *Anisakis* excretory-secretory products is responsible for the polyclonal IgE stimulation. To determine whether this clinical entity can help us to elucidate the biologic and immunologic relevance of sCD23 and IL-4, we investigated the relationship between the cytokines sCD23 and IL-4, on the one hand, and specific and total IgE, on the other hand, in this acute parasitism, with a followup over 6 months.

Material and methods

Study population

Patients were selected by the criteria of high suspicion of gastroallergic anisakiasis: patients arrived at the emergency room with acute urticaria/angioedema or anaphylaxis. They were asked for further abdominal symptoms such as upper abdominal pain, nausea, or vomiting. If they had eaten raw or undercooked fish in the 24 h prior to the initiation of allergic symptoms, a first serum sample was

taken and stored at -70°C . In nine patients, fiber-optic gastroscopy was performed, detecting a live larva of *A. simplex* in two of them; in four patients, we found signs of recent mucosal damage by the supposed parasite. If a skin prick test (SPT) with *A. simplex* was positive in an allergologic evaluation within 1 week, patients were diagnosed as having gastroallergic anisakiasis (SPT were carried out with *A. simplex* antigen (Lab. IPI, Madrid, Spain) and were considered positive with a mean wheal diameter of $\geq 3 \times 3$ mm; histamine at 1% concentration and saline solution (0.9% NaCl) were the positive and negative controls, respectively). Thus, 16 patients were included for followup: eight male and eight female with a mean age of 46.3 (SD 17.2) years. In this group, 13/16 patients had urticaria, nine had angioedema, and three had low blood pressure (systolic arterial pressure of <100 cm Hg). Four of them had previously had similar symptoms related to fish intake, and three patients had previously had acute urticaria or angioedema of unknown origin. In all but two patients, symptoms disappeared within 24 h; one patient needed antihistamines for 3 days, and one patient needed antihistamines and corticosteroids for 2 weeks. All patients were advised to avoid raw or undercooked fish and remained asymptomatic after the acute episode. Serum samples were taken in the emergency room or as soon as possible (day 0; $n=16$) and after 1 month (day 30; $n=16$) and 6 months (6 months; $n=10$).

Assays for specific IgE, total IgE, IL-4, and sCD23

Total IgE was assessed by the IMx-Method (Abbot Diagnostics, Chicago, IL, USA). Specific IgE against *A. simplex* was assessed by CAP-FEIA (Pharmacia, Uppsala, Sweden). Specific IgE was considered positive with results of >0.35 kU/l.

sCD23 and IL-4 were assessed by ELISA according to the manufacturer's assay procedure (Cellfree[®] Human ELISA sCD23 and Human ELISA IL-4, Endogen, Woburn, MA, USA).

Statistics

Mean values were calculated for sCD23, and day 0 values were compared with day 30 values by *t*-test for paired samples. Median values were calculated for specific and total IgE, and day 0 values were compared with day 30 values by the Wilcoxon matched-pairs signed-ranks test. Pearson correlation coefficients were calculated for sCD23 and for specific and total IgE after ln-transformation. No correlation studies were performed with IL-4 because of the low number of positive samples.

Results

Specific and total IgE

Median specific IgE rose from 10.8 (interquartile range [IQR] 7.2–46.8) kU/ml to 35.8 (30.8–91.8) kU/ml and afterward fell to 24.9 (14.5–87.0) kU/ml ($P<0.05$). Median total IgE rose from 71 (IQR 46–209) kU/ml to 271 (111–988) kU/ml and fell to 115.0 (66–461) kU/ml ($P<0.07$) (Fig. 1A and B). In 15 of 16 patients, specific IgE increased after 1 month; in 14 of 16 patients, total IgE increased. In these patients, the mean percentual increment for specific IgE was 353% (SD 340%); for total IgE, it was 396% (SD 410%).

sCD23

The mean value for sCD23 at day 0 was 28.6 (SD 11.3) U/ml, with no significant changes after 1 or 6 months

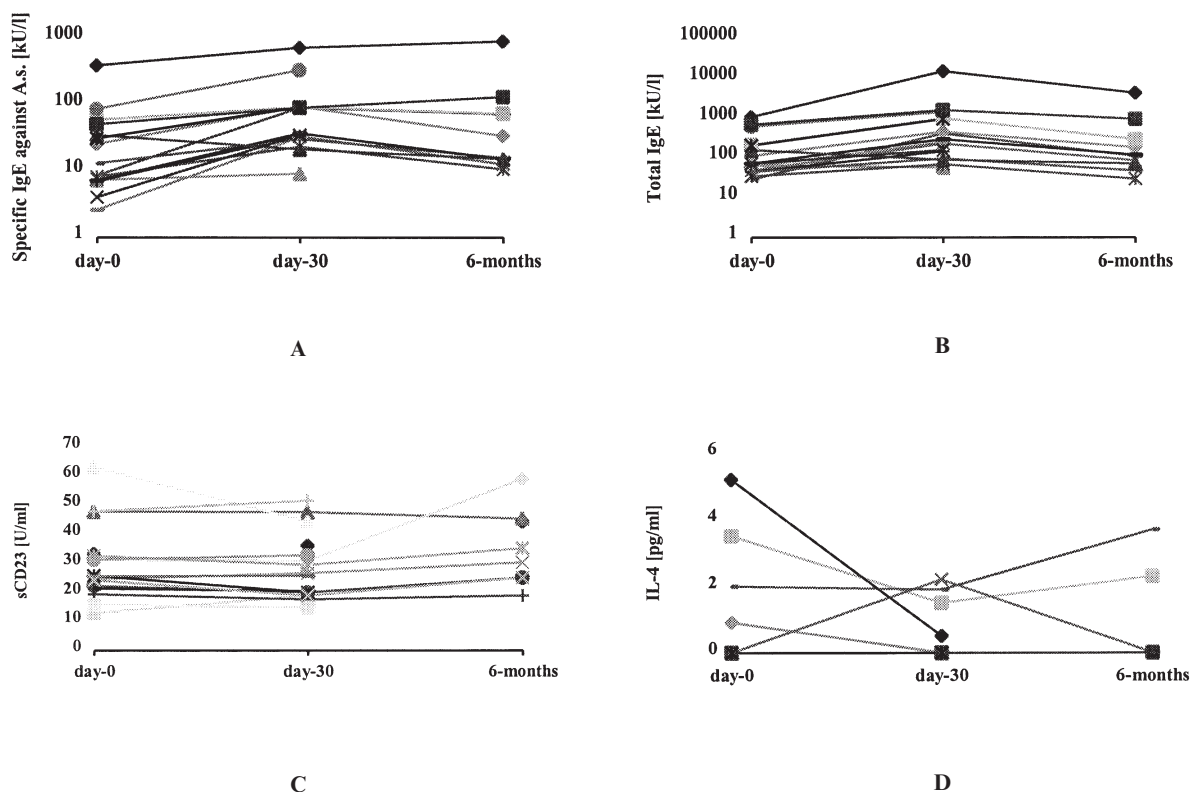


Figure 1. A–D) Follow-up of sCD23, IL-4, specific IgE against *Anisakis simplex* (A.s.), and total IgE. IL-4 was detectable in only four (4/2) serum samples of day 0 (day 30/6 months). No significant changes were detected for sCD23, but significant increase was observed for most specific IgE and total IgE values after 1 month.

Table 1. Studies of correlation between sCD23, specific IgE, and total IgE

	Specific IgE			Total IgE		
	Day 0	Day 30	6 months	Day 0	Day 30	6 months
sCD23						
<i>r</i>	0.61	0.55	0.69	0.42	0.54	0.62
<i>P</i>	0.013	0.026	0.026	0.102	0.030	0.056
	Ratio (specific IgE/total IgE)			Increment of specific IgE	Increment of total IgE	
	Day 0	Day 30	6 months			
sCD23						
<i>r</i>	0.56	−0.25	0.03	−0.28	0.56	
<i>P</i>	0.024	0.36	0.93	0.31	0.038	

Pearson correlation coefficients (*r*) and significance (*P*). sCD23 values at day 0. Specific and total IgE were ln-transformed. Percentual increments were calculated for day 30 values with regard to day 0 values.

(Fig. 1C). Correlation factors are outlined in Table 1. There was a positive correlation between sCD23 at day 0 and specific IgE at day 0 and followup, as well as between sCD23 at day 0 and total IgE values. There was a positive correlation between the ratio of specific IgE to total IgE at day 0 and sCD23. Together with the finding of a positive correlation between sCD23 and the percentual increment of total IgE, but not of specific IgE, this indicates that sCD23 is probably more related to the amount of total IgE than to the amount of specific IgE.

IL-4

Only 4/16 serum samples at day 0 showed measurable IL-4 levels, ranging from 0.9 to 5.2 pg/ml, 4/16 serum samples after 1 month (0.5–2.2 pg/ml), and 2/10 serum samples after 6 months (2.3–3.7 pg/ml) (Fig. 1D).

Discussion

CD23, which is the low-affinity receptor for IgE FcεRII, is a C-type lectin on the surface of various hematopoietic cells, including B and T cells, monocytes, eosinophils, and platelets. The soluble cytokine sCD23 is produced by an autocatalytic cleavage of surface CD23 into various fragments, a 25-kDa molecule being the most stable form. At the molecular level, sCD23 has been found to be a hallmark in the regulation of IgE synthesis, and IL-4 is the most potent inducer of CD23 expression. However, the relationship between serum IL-4, serum sCD23, and IgE has been controversial in clinical studies.

This is partly due to the large individual variability of sCD23, IL-4, and total IgE values (14–16), but also to the different study designs. Different studies have shown sCD23 to be elevated in atopic subjects, but also in helminthic infestations, both circumstances in

which high IgE levels can be detected. We should therefore expect to observe a positive correlation between sCD23 and IgE, a correlation that has been confirmed in only a few studies. A recent study of pollen-allergic patients, found a positive correlation between sCD23 and total IgE independently of their clinical symptoms (rhinitis or asthma; during the pollen season or out of it) (16). In another study of pollen-allergic patients, the seasonal increase in total and specific IgE was not accompanied by a similar change in sCD23 values (17). Other studies including atopic patients of various clinical conditions and ages could not find any correlation between sCD23 and IgE (18); however, one study detected an inverse correlation of these two factors in asthmatic patients (19). Another study of chronically parasitized children found a negative correlation between sCD23 and total IgE and explained it by the stabilizing effect of IgE on cell-surface CD23 (20). All these studies were based on an interindividual comparison, so that, taking into account the large individual variability of the cytokines and IgE, it would be more illuminating to study the relationship of sCD23 and IgE in intraindividual comparisons. In this respect, our study helps to clarify the relationship of sCD23 and IgE, as we deal with a longitudinal human study design that resembles experimental animal studies. Because of the peculiar clinical signs discussed elsewhere (8, 9), we were able to recruit patients with an acute allergic type-I hypersensitivity reaction due to the contact of the nematode *A. simplex* with the gastric mucosa, so that we know the exact moment of infestation with the larvae, a circumstance that can only be compared with animal experiments. However, we now know that gastroallergic anisakiasis is a mostly self-limited disease, as man is only a “dead-end” host, and chronic forms of anisakiasis are infrequent (21, 22).

sCD23 levels were not elevated in our study population if we compare them with reference values or control patients of other studies (16, 23). We found an important interindividual variability, but could not detect an intraindividual variation in a 6-month followup. This is in contrast to the important changes in total and specific IgE where we confirmed an increase of specific and total IgE after 1 month and a gradual decrease over 6 months afterward, as has been shown elsewhere (24).

However, we easily confirmed a positive correlation between sCD23 and specific and total IgE values. As sCD23 values remain stable over a period of 6 months after the acute reaction and without any influence of residual parasite molecules after, at most, a few days, it seems that these correlations are independent of the action of the parasite. This is also corroborated by the fact that a significant correlation can also be found between these parameters in serum samples obtained in the first hours after the acute reaction, where IgE values are supposed to be at baseline levels.

An interesting feature of our study that has not been reported previously is the positive correlation of sCD23 with the percentual increment of total IgE. This means that independently of the basal total IgE level in an individual, higher levels of sCD23 predispose to a larger production of total IgE, but not specific IgE. This has to be interpreted as sCD23 influencing the polyclonal expansion of IgE-producing B cells. sCD23 enhances *in vitro* the production of IgE at an IL-4-independent stage, suggesting that it regulates the activity of B cells after switching to IgE (1). This could confirm our findings, as IgE antibodies with unrelated specificities are enhanced in accordance with the sCD23 levels.

In our study design, we expected to find sCD23 levels not only to correlate with IgE, but also to be time-related to the action of the parasite. The first serum sample was obtained in the first 24 h after the beginning of the acute allergic reaction, and we cannot rule out that sCD23 could have been found to be elevated at a very early time or between the taking of this early sample and the second after 1 month. However, the fact that in three consecutive samples over a time span of 6 months sCD23 levels remained nearly the same renders unlikely the idea that our values were influenced by the action of the parasite.

It has been proposed that parasites may secrete molecules with activities or structures homologous to cytokines such as IFN- γ , sCD23, or IL-4 (25–27). Moreover, *A. simplex* antigens share cross-reactive epitopes with the host cytokine IL-4, a fact that leads to the assumption of a possible biologic significance (28). Another factor is Der p 1, a major allergen of the house-dust mite, which has been shown to cleave specifically

CD23 (12, 13). Our results do not support one of these mechanisms if these were to be detected with changes of IL-4 or sCD23 in serum. Further studies should therefore concentrate on local changes or take into account other possible time kinetics.

Like sCD23, IL-4 has also been reported to be elevated in atopics and parasitic diseases (18, 29). IL-4, arguably the paradigmatic type-2 cytokine, is the most important cytokine mediating IgE synthesis (30). The biology of allergic diseases closely parallels that of IL-4 and IL-13. IL-4 is the most potent inducer of CD23 expression (1). In our study, we did not find elevated levels of IL-4 at any time. This is explained by the fact that our patients did not belong either to the group of chronic helminthiasis, or to an atopic group (data not shown). Nevertheless, it was surprising not to find elevated levels even in this peculiar acute parasitism, in which the larvae are able to induce high levels of total and specific IgE. Probably locally produced or secreted IL-4 was not high enough to be detectable in serum at day 0 or after 1 month, but it is also possible that we were not able to detect the right moment of IL-4 elevation.

In conclusion, the high amplification of specific IgE against *A. simplex* and of total IgE after an acute contact with the nematode was not accompanied by measurable changes in serum sCD23 or IL-4. The present findings suggest that in gastroallergic anisakiasis it is not the parasite that acts on serum sCD23 and IL-4 values; rather, the interindividual variability of basal sCD23 determines the amount of specific and total IgE and, what is more interesting, the amount of polyclonal expansion of IgE.

References

1. DELESPESE G, SARFATI M, WU CY, FOURNIER LETELIER M. The low-affinity receptor for IgE. *Immunol Rev* 1992;**125**:77–97.
2. ROMAGNANI S. Human Th1 and Th2 subsets: doubt no more. *Immunol Today* 1991;**12**:256–257.
3. FINKELMAN FD, PEARCE EJ, URBAN JF, SHER A. Regulation and biological function of helminth-induced cytokine responses. *Immunol Today* 1991;**12**:A62–A66.
4. FINKELMAN FD, SHEA-DONAHUE T, GOLDHILL J, et al. Cytokine regulation of host defense against parasitic gastrointestinal nematodes: lessons from studies with rodent models. *Annu Rev Immunol* 1997;**15**:505–533.
5. SUTTON BJ, GOULD HJ. The human IgE network. *Nature* 1993;**366**:421–428.
6. YU P, KOSCO-VILBOIS M, RICHARDS M, KÖHLER G, LAMERS MC. Negative feedback regulation of IgE synthesis by murine CD23. *Nature* 1994;**369**:753–756.
7. ALONSO A, DASCHNER A, MORENO-ANCILLO A. Anaphylaxis with *Anisakis simplex* in the gastric mucosa. *N Engl J Med* 1997;**337**:351–352.
8. DASCHNER A, ALONSO-GÓMEZ A, CABALLERO MT, et al. Gastroallergic anisakiasis: an underestimated cause of acute urticaria and angioedema? *Br J Dermatol* 1998;**139**:822–828.
9. DASCHNER A, ALONSO-GÓMEZ A, CABAÑAS R, SUAREZ-DE-PARGA JM, LÓPEZ-SERRANO MC. Gastroallergic anisakiasis: borderline between food allergy and parasitic disease – clinical and allergological evaluation of 20 patients with confirmed acute parasitization by *Anisakis simplex*. *J Allergy Clin Immunol* 2000;**105**:176–181.
10. DASCHNER A, CUÉLLAR C, PASCUAL CY, SÁNCHEZ-PASTOR S, ALONSO-GÓMEZ A, MARTÍN-ESTEBAN M. Immunoblotting patterns in patients with gastroallergic anisakiasis depend on the time elapsed between the allergic reaction and obtaining of serum sample [Abstract]. *J Allergy Clin Immunol* 2000;**105**:S43.
11. DASCHNER A, ALONSO-GÓMEZ A, CABALLERO MT, SUÁREZ-DE-PARGA JM, LÓPEZ-SERRANO MC. Usefulness of early serial measurement of specific and total IgE in the diagnosis of gastroallergic anisakiasis. *Clin Exp Allergy* 1999;**29**:1260–1264.
12. SCHULZ O, LAING P, SEWELL HB, SHAKIB F. Der p 1, a major allergen of the house dust mite, proteolytically cleaves the low-affinity receptor for human IgE (CD23). *Eur J Immunol* 1995;**25**:3191–3194.

13. HEWITT CRA, BROWN AP, HART BJ, PRITCHARD DI. A major house dust mite allergen disrupts the immunoglobulin E network by selectively cleaving CD23: innate protection by antiproteases. *J Exp Med* 1995;**182**:1537–1544.
14. BOCCAFOGLI A, VICENTINI L, LAMBERTINI D, SCOLOZZI R. Soluble CD23 is increased in allergy. *Allergy* 1997;**52**:357–358.
15. REDDY MM, WEISSMANN AM, MAZZA DS, et al. Circulating elevated levels of soluble CD23, interleukin-4, and CD20+ CD23+ lymphocytes in atopic subjects with elevated serum IgE concentrations. *Ann Allergy* 1992;**69**:131–134.
16. DI LORENZO G, DRAGO A, PELLITTERI ME, et al. Serum levels of soluble CD23 in patients with asthma or rhinitis monosensitive to *Parietaria*. Its relation to total serum IgE levels and eosinophil cationic protein during and out of the pollen season. *Allergy Asthma Proc* 1999;**20**:119–125.
17. YANIGAHARA Y, SARFATI M, MARSH D, NUTMAN T, DELESPESE G. Serum levels of IgE-binding factor (soluble CD23) in diseases associated with elevated IgE. *Clin Exp Allergy* 1990;**20**:395–401.
18. OSHIMA Y, KATAMURA K, MIURA M, MIKAWA H, MAYUMI M. Serum levels of interleukin 4 and soluble CD23 in children with allergic disorders. *Eur J Pediatr* 1995;**154**:723–728.
19. SHAKIB F, BOULSTRIDGE L, SMITH SJ. A study of the interrelationship between circulating IgG subclass anti-IgE autoantibodies, IgE and soluble CD23 in asthma. *Allergol Immunopathol (Madr)* 1993;**21**:20–24.
20. PRITCHARD DI, KUMAR S, EDMONDS P. Soluble (s)CD23 levels in a parasitized population from Papua New Guinea. *Parasite Immunol* 1993;**15**:205–208.
21. HSIU JG, GAMSEY AJ, IVES CE, DAMATO NA, HILLER AN. Gastric anisakiasis: report of a case with clinical, endoscopic and histological findings. *Am J Gastroenterol* 1986;**81**:1185–1187.
22. LÓPEZ-SERRANO MC, ALONSO-GÓMEZ A, MORENO-ANCILLO A, DASCHNER A, SUÁREZ DE PARGA JM. Gastroallergic anisakiasis: immediate hypersensitivity due to *Anisakis simplex* infestation. *Allergol Immunol Clin* 2000;**15**:230–236.
23. Endogen Cellfree Human ELISA sCD23. Manufacturer's instructions. Performance Characteristics. 5. Expected values. Endogen, Inc., 1997.
24. DEARDORFF TL, KAYES SG, FUKUMARA T. Human anisakiasis transmitted by marine food products. *Hawaii Med J* 1991;**50**:9–16.
25. PRITCHARD DI. Immunity to helminths: is too much IgE parasite- rather than host-protective? *Parasite Immunol* 1993;**15**:5–9.
26. GRENCIS RK, ENTWISTLE GM. Production of an interferon-gamma homologue by an intestinal nematode: functionally significant or interesting artefact? *Parasitology* 1997;**115**:S101–S105.
27. LEE TDG, XIE CY. IgE regulation by nematodes: the body fluid of *Ascaris* contains a B-cell mitogen. *J Allergy Clin Immunol* 1995;**95**:1246–1254.
28. CUÉLLAR C, PERTEGUER MJ, RODERO M. Presence of IL-4-like molecules in larval excretory-secretory products and crude extracts from *Anisakis simplex*. *Scand J Immunol* 2001;**53**:483–488.
29. LYNCH NR, HAGEL I, PEREZ M, DI PRISCO MC, LOPEZ R, ALVAREZ N. Effect of anthelmintic treatment on the allergic reactivity of children in a tropical slum. *J Allergy Clin Immunol* 1993;**92**:404–411.
30. KOBAYASHI H, ISHIZUKA T, OKAYAMA Y. Human mast cells and basophils as sources of cytokines. *Clin Exp Allergy* 2000;**30**:1205–1212.