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Specific and total IgE in patients with recurrent, acute urticaria caused by *Anisakis simplex*

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Titres of parasite-specific IgE were investigated in 19 patients thought to have recurrent, acute urticaria caused by sensitization to *Anisakis simplex* (Dujardin, 1845), before and after they were placed on a fish-free diet. Patients with other allergic disease and those being treated with corticosteroids or antihistaminics were excluded.

Skin-prick tests were carried out with *A. simplex* extract, and blue- and white-fish extracts. The CAP system (Pharmacia), a commercial test kit developed for the assay of food-specific IgE, was used to monitor serum concentrations of total IgE and antigen-specific IgE against *Anisakis*, *Ascaris*, *Echinococcus*, *Toxocara*, tuna, salmon, shrimp, mussel and cod. Before going on a fish-free diet, the 19 patients had CAP scores against *A. simplex* of 5 (three cases), 3 (seven) or 2 (nine). After a mean of 120 days on the diet, the scores against *A. simplex* were unchanged in 15 of the cases, reduced in three [from 5 to 4 (one case) or from 2 to 0 (two cases)] and increased in one (from 2 to 3). Most (16) of the patients no longer had any urticaria and the others reported significant reductions in the intensity and frequency of their symptoms.

Human anisakiasis is a gastric, intestinal or ectopic disease (Ishikura *et al.*, 1993) caused by larval nematodes of the family Anisakidae, especially those of *Anisakis simplex*. The infestation is acquired by eating raw or undercooked fish or squid (Sakanari and McKerrow, 1989). The vagueness of its symptoms means that anisakiasis is often misdiagnosed, as appendicitis, acute abdominal pain, gastric tumour or cancer, ileitis, cholecystitis, diverticulitis, tuberculous peritonitis, cancer of the pancreas, or Crohn's disease (Sakanari and McKerrow, 1989). The acute symptoms may be caused by a type-I allergic reaction in the gastro-intestinal wall (Suzuki *et al.*, 1970, 1975), with elevated specific IgE after the onset of clinical symptoms (Yagihashi *et al.*, 1990). However, Kasuya *et al.* (1990) observed that *Anisakis* larvae were the real causative agents in some patients who had urticaria but not abdominal pain or any other clinical indication of anisakiasis.

Although the first Spanish case of sensitization attributed to *Anisakis simplex* (Dujardin, 1845) was only reported 5 years ago (Audícana *et al.*, 1995), several other such cases have since been observed and investigated (Fernández de Corres *et al.*, 1996; Montoro *et al.*, 1997; Anibarro and Seoane, 1998; Armentia *et al.*, 1998; Cuende *et al.*, 1998; Daschner *et al.*, 1998; Fraj *et al.*, 1998; Rosel *et al.*, 1998; Alonso *et al.*, 1999; García-Labairu *et al.*, 1999; Mendizabal-Basagoiti, 1999). Since the Spanish consume large quantities of fish (78.2 g/person.day), the real prevalence

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and incidence of anisakiasis in Spain may be much greater than indicated by these reported cases.

Urticaria is a very common allergic disease, with a cumulative prevalence of 15%–25% among allergic individuals (Meynadier and Meynadier, 1990). Most cases of chronic and recurrent, acute urticaria have been labelled idiopathic, with no causative factor being found in up to 70% of cases. In approximately 50% of cases, hives are associated with angio-oedema, presenting as swelling of the subcutaneous tissues. The identification of any orally ingested agent as the cause of urticaria may be difficult. Occult, but clinically relevant allergens may be present in a patient's diet. For example, the acute recurrent urticaria observed in some patients who usually eat fish may be the result of sensitization to a common parasite of fish (*An. simplex*) rather than to the fish itself (Montoro *et al.*, 1997).

Sensitization to *An. simplex* may reflect previous anisakiasis or repeated exposure to larval antigens. It is not certain that all reactions to *An. simplex* are the result of the generation of specific immune responses to the organism or of cross-reactivity with other antigens to which a potential host has been exposed. This uncertainty has implications for the predictive value of diagnostic assays. Furthermore, the protective/pathogenic roles of IgE in this sensitization remain to be determined. To try to throw some light on these topics, serum concentrations of total IgE and parasite-specific IgE were investigated in a group of patients who appeared to be sensitized to *An. simplex*.

PATIENTS AND METHODS

Patients

The 19 patients investigated (11 females and eight males, aged 13–78 years) were residents of Madrid, and had presented at the Immunology and Allergy Service of the Hospital del Aire (Madrid) with recurrent, acute urticaria. All had been found seropositive for specific IgE to *An. simplex* (Montoro *et al.*, 1997). During the present study, each was put on a fish-free diet. Patients in whom any other allergic disease had been diagnosed and those being treated with corticosteroids or antihistaminics were excluded.

Skin Test

A commercial extract of *An. simplex* (1 mg/ml; International Pharmaceutical Immunology, ASAC Pharmaceutical International, Alicante, Spain) was used to investigate each patient's sensitization to the parasite, using prick-tests on the skin of the volar forearm. The observation, 15 min later, of a wheal measuring at least 3 mm in diameter was considered indicative of a positive result. Histamine (10 mg/ml) and saline were used as positive and negative controls, respectively. Commercial skin tests (ALK; Abelló Farmacia, Madrid) of sensitization to blue fish [sea bream *(Pagellus centrodontus)*, anchovy (*Engraulis encrasicolus*), and red mullet (*Sardina pilchardus*)] and white fish [cod (*Gadus morrhua*), common sole (*Solea solea*), common bass (*Roccus labras*), and hake (*Merluccius merluccius*)] were also performed.

Determination of IgE

Serum concentrations of total IgE and of IgE against *Anisakis*, *Ascaris*, *Echinococcus*, *Toxocara*, tuna, salmon, shrimp, mussel or cod were determined using the CAP system (Pharmacia & Upjohn, Uppsala), a commercial immuno-assay (Leimgruber *et al.*, 1991). The concentrations of IgE detected were converted to CAP 'scores' of 0 (<0.35 kU/litre), 1 (0.35–0.7 kU/litre), 2 (0.7–3.5 kU/litre), 3 (3.5–17.5 kU/litre), 4 (17.5–50 kU/litre), 5 (50–100 kU/litre) or 6 (>100 kU/litre). According to the manufacturer's instructions, concentrations of specific IgE >0.35 kU/litre (i.e. a CAP score of 1 or more) should be considered positive. In an attempt to avoid false-positive results, however, the threshold was set twice as high, at 0.70 kU/litre (i.e. a CAP score of 2). IgE concentrations in sera collected on presentation and on follow-up, after 12–214 days on the fish-free diet, were compared statistically, using Wilcoxon tests and commercial statistical software (SPSS version 8.0; SPSS Inc, Chicago, IL).

RESULTS AND DISCUSSION

The 19 patients studied developed urticaria each time they ingested fish or other seafood. Although 15 of them gave a positive response in the *Anisakis* skin-prick test (Fig. 1), none reacted to any of the fish extracts used in the prick tests.

All 19 patients were seropositive, on presentation, for IgE against *Anisakis*, with CAP scores of 5 (three patients), 3 (seven) or 2 (nine). After varying times on a fish-free diet (with a mean of 120 days), 15 of the 19 patients showed no change in their scores (Fig. 1). Although three patients showed a reduction, either from a score of 5 to one of 4 (one patient) or from 2 to 0 (two patients), and one showed an increase (from as score of 2 to one of 3), none of these changes was statistically significant.

In terms of symptomology, three patients reported an important reduction in the intensity and frequency of their symptoms and none of the rest reported any urticaria after a few months on a fish-free diet. The present results therefore indicate that removal of (marine) fish from the diet of patients sensitized to *An. simplex* is enough to ameliorate or eliminate their recurrent, acute urticaria. However, the reductions seen in the serum concentrations of parasite-specific (Fig. 1) and total IgE (Fig. 2) were not statistically significant.

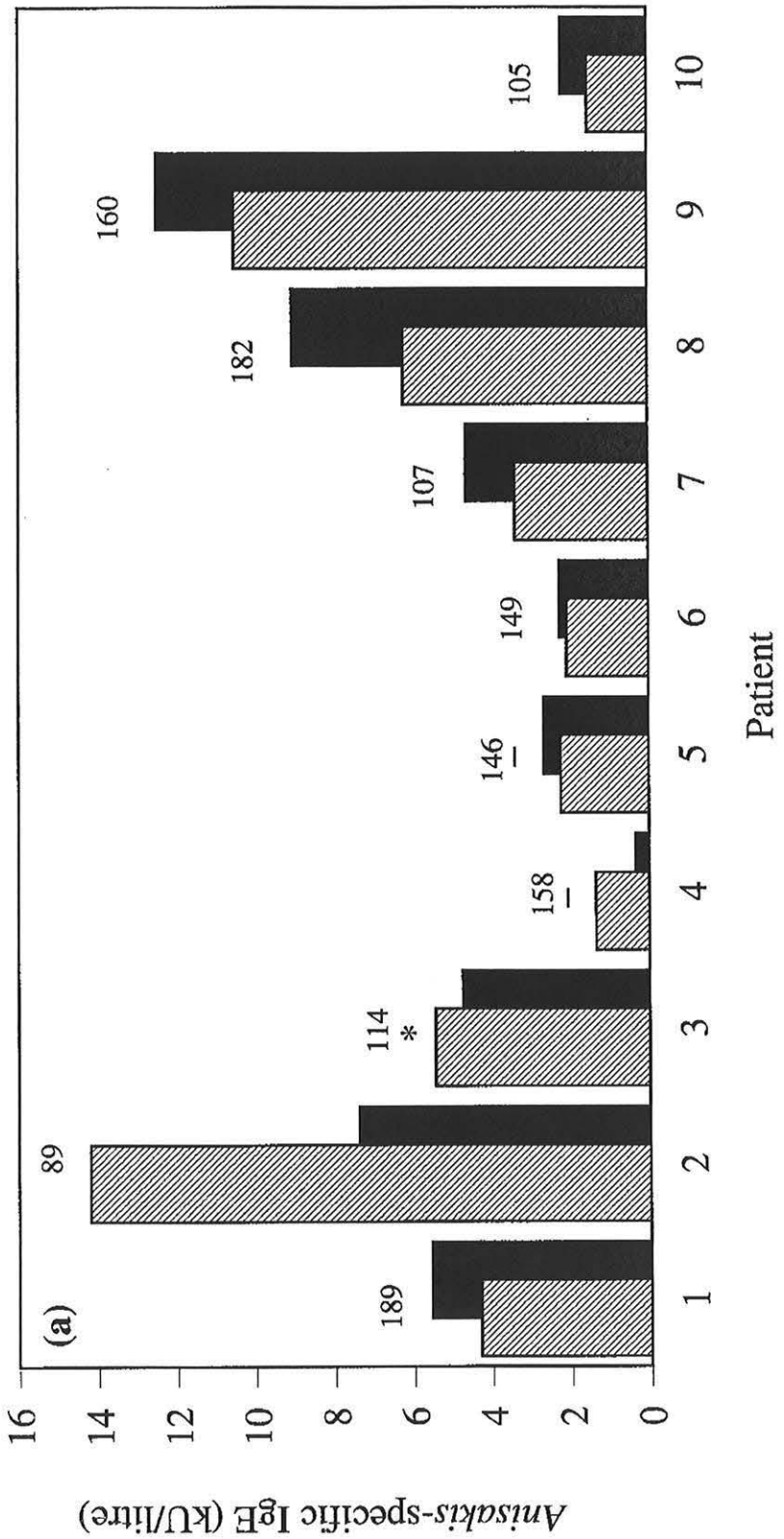
Four of the 19 patients investigated were prick-test negative for sensitization to *Anisakis* (Fig. 1). Two of these four (patients 4 and 5) were only just positive for *Anisakis*-specific IgE on presentation (with a CAP score of 2) and were negative for this IgE at follow-up. One of the other two (patient 17) had a higher anti-*Anisakis* score on presentation (3) and slight cross-reactivity with *Ascaris* (with a CAP score of 1). The other (patient 19) had a CAP score of 2 for anti-*Anisakis* IgE and gave positive reactions to salmon and shrimp at presentation.

Patient 3 had apparent serum concentrations of *Ascaris*-specific IgE that were lower than those of anti-*Anisakis* IgE. In contrast, Sakanari *et al.* (1988) observed that, in a patient with confirmed ascariasis, the apparent concentrations of anti-*Anisakis* IgE (determined by radio-allergosorbent tests) were greater than those of anti-*Ascaris* IgE. Obviously, any cross-reactivities between *Anisakis* and *Ascaris* antigens could invalidate the diagnosis of sensitization to *Anisakis*, if this is based solely on the apparent concentration of anti-*Anisakis* IgE.

In 12 of the 19 patients, changes in the concentrations of total IgE between presentation and follow-up were matched by changes in anti-*Anisakis* IgE. This is more noticeable when actual concentrations (kU/litre) are studied than when CAP scores are compared. For instance, although patient 1 had an anti-*Anisakis* CAP score of 3, both at presentation and follow-up, there were slight increases in the serum concentrations of anti-*Anisakis* IgE (4.29 *v.* 5.54 kU/litre) and total IgE (23.8 *v.* 46.3 kU/litre) over this period. Conversely, although patient 3 also scored 3 for anti-*Anisakis* IgE at both determinations, the actual concentrations of anti-*Anisakis* IgE (5.43 *v.* 4.73 kU/litre) and of total IgE (635 *v.* 4.61 kU/litre) fell while this patient was on a fish-free diet.

Two patients bucked this trend. In patient 13, levels of anti-*Anisakis* IgE increased, from 77 to 80.5 kU/litre, while those of total IgE decreased, from 1254 to 444 kU/litre. Conversely, in patient 17, specific IgE decreased, from 8.11 to 6.35 kU/litre, while total IgE increased from 175 to 189 kU/litre. However, since patient 13 appeared seropositive for *Anisakis*, *Ascaris* and *Echinococcus*, and patient 17 (who was prick-test negative) appeared positive for *Anisakis* and *Ascaris*, the differences between specific-IgE and total-IgE responses could be due to other infections rather than *Anisakis* sensitization.

The debate on the relative protective roles of specific and total IgE is often related to infections with parasitic nematodes. Since the identification of reaginic antibodies in animals infected with parasites (Ogilvie, 1964), their role in defence against parasitic infections has been discussed by several workers. Capron *et al.* (1977, 1986) and Capron and Capron (1986) observed the protective role of specific


(a)
189
89
114
*
158
—
146
—
149
107
182
160
105
1
2
3
4
5
6
7
8
9
10
Patient
0
2
4
6
8
10
12
14
16
Anisakis-specific IgE (kU/litre)

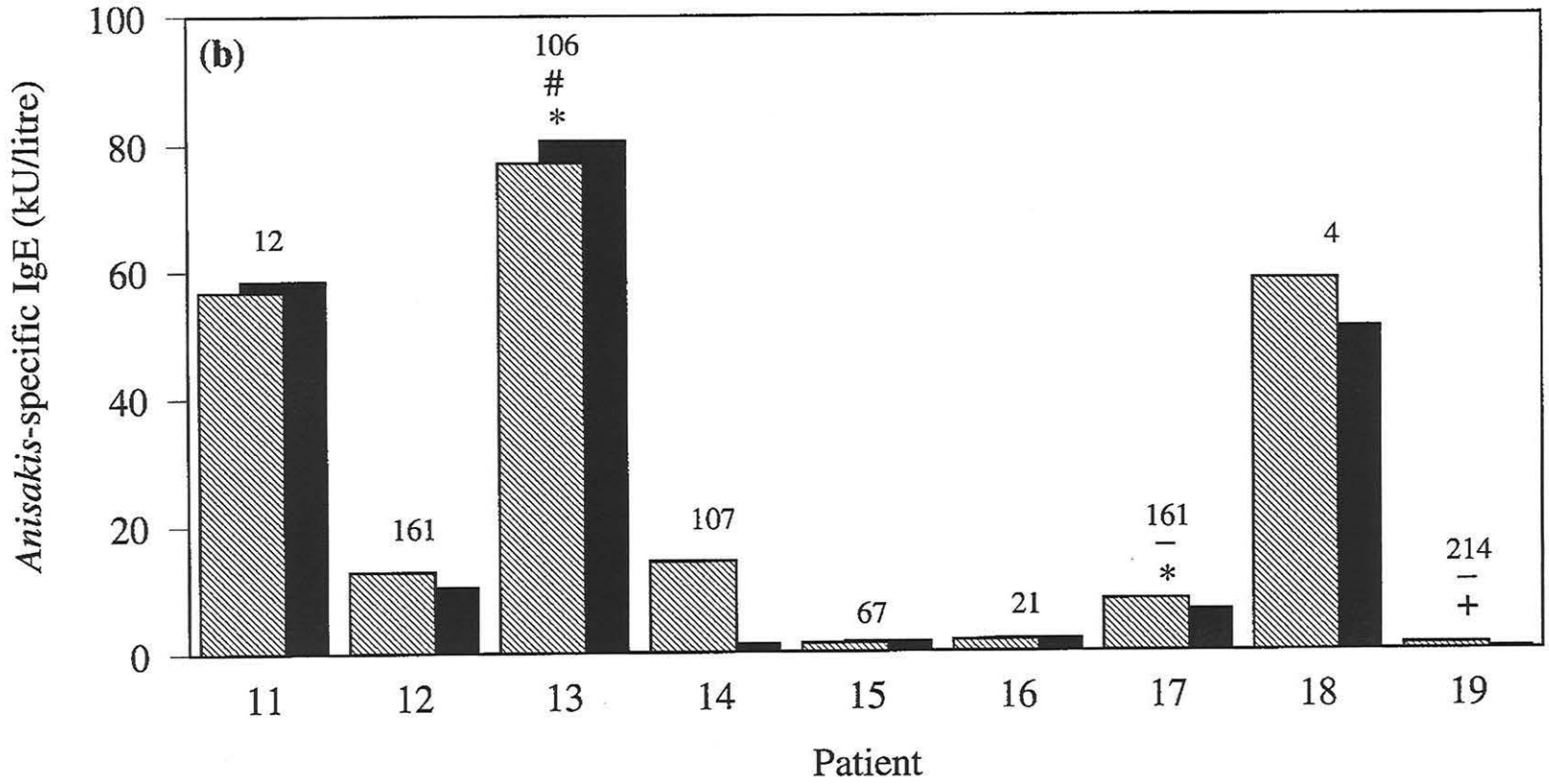
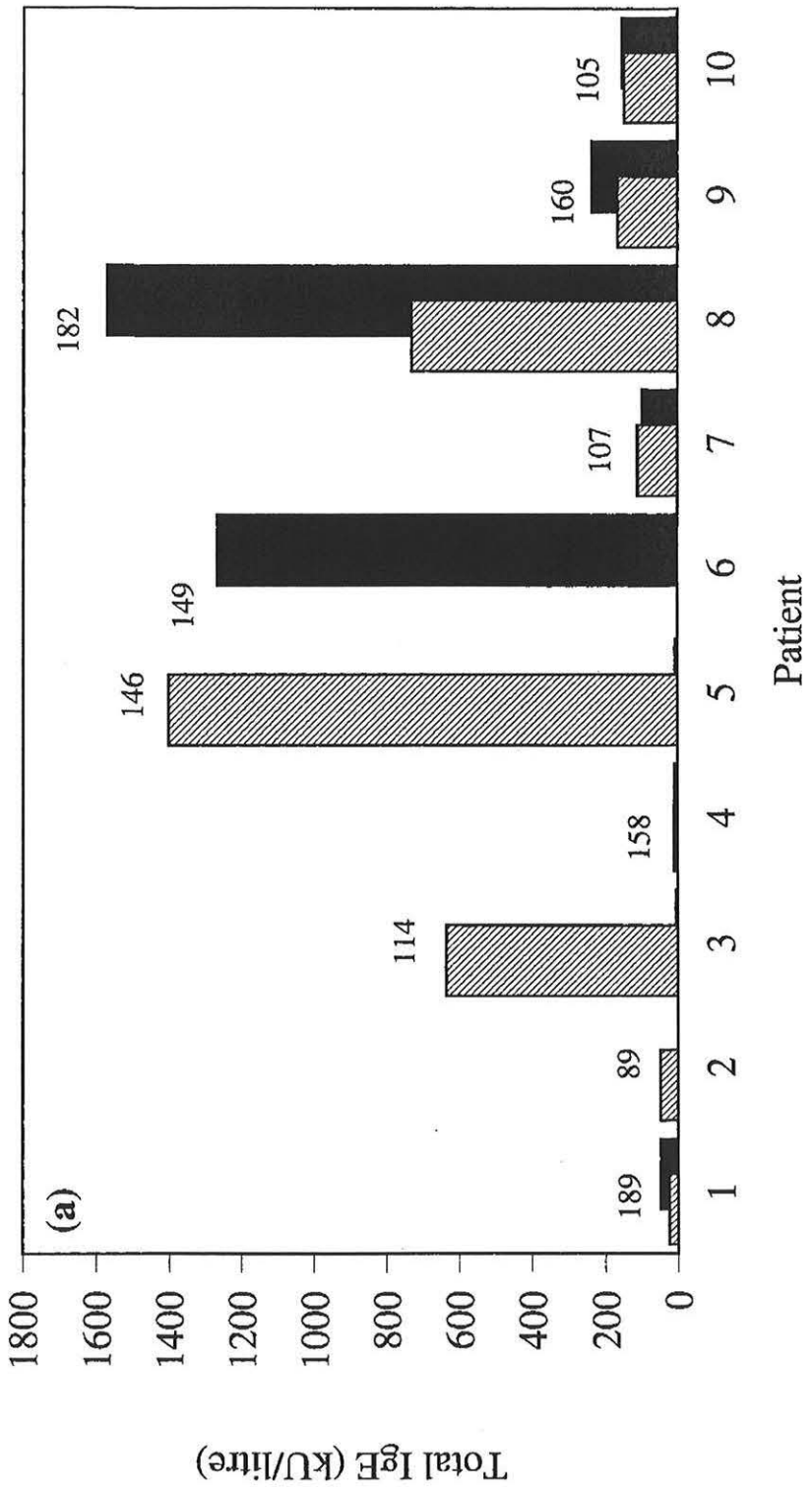


Fig. 1. Concentrations of IgE against *Anisakis simplex* in sera, from patients 1–10 (a) and 11–19 (b), collected at presentation (▨) and follow-up (■). The number above each pair of bars indicates the time, in days, between the two determinations. The patients found to be prick-test-negative for *An. simplex* (–) or to be positive for *Ascaris*-specific (*), *Echinococcus*-specific (#) or salmon- and shrimp-specific (+) IgE are indicated.


(a)
Total IgE (kU/litre)
Patient
1800
1600
1400
1200
1000
800
600
400
200
0
1
2
3
4
5
6
7
8
9
10
189
89
114
158
146
149
107
182
160
105

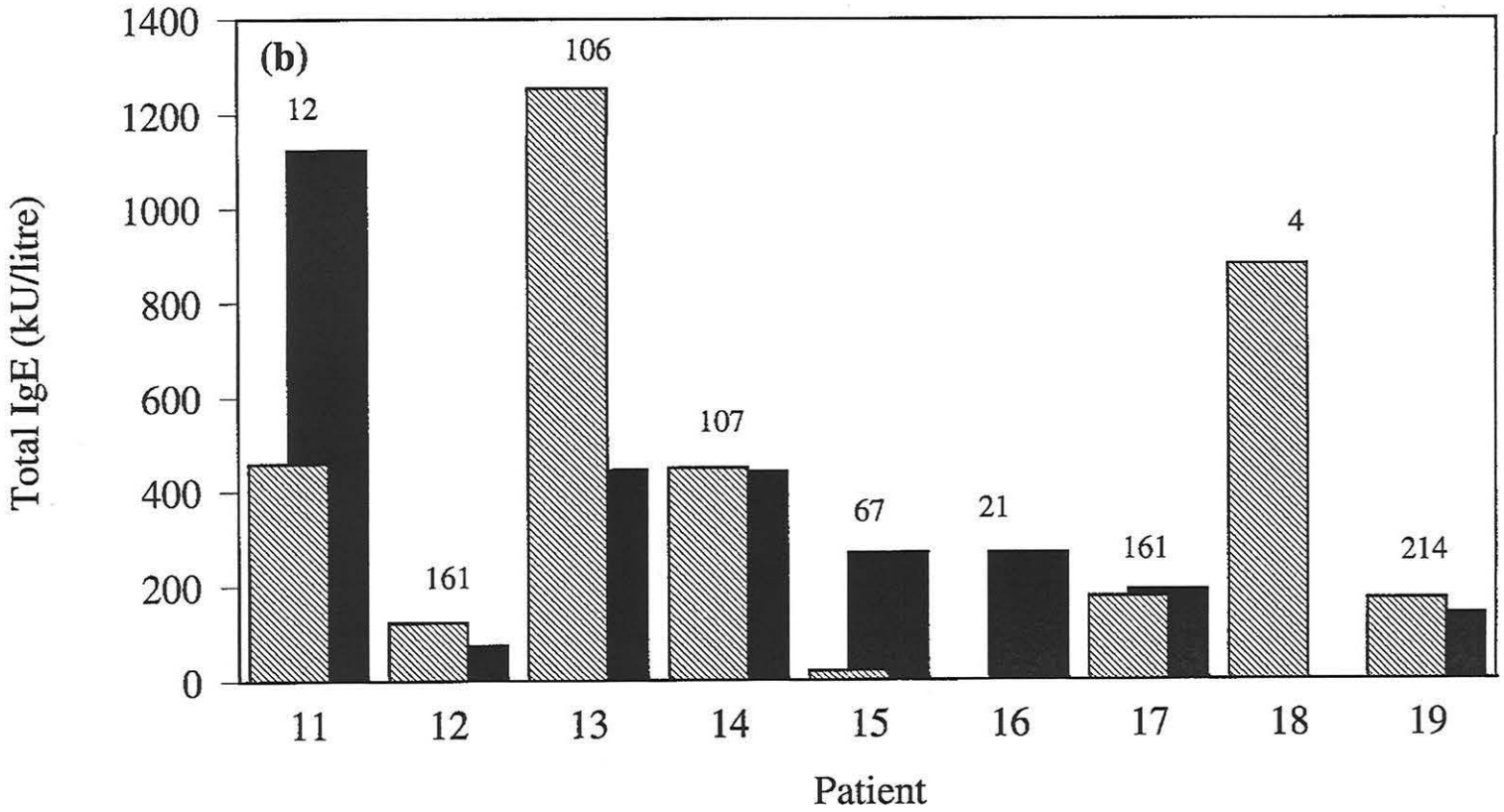


Fig. 2. Concentrations of total IgE in sera, from patients 1–10 (a) and 11–19 (b), collected at presentation (▨) and follow-up (■). The number above each pair of bars indicates the time, in days, between the two determinations.

IgE in *Schistosoma mansoni* infecton. An association is known to exist between high levels of specific IgE and resistance to re-infection with *Sc. haematobium* or *Sc. mansoni* (Hagan *et al.*, 1991; Rihet *et al.*, 1992; Dunne and Pearce, 1999). The components of a conventional allergic response could potentially contribute to the immune defence against helminth infections. Conversely, total IgE induced by parasitic helminths could help the parasites survive (Capron and Dessaint, 1975). Total IgE might be in competition with specific IgE and so favour the parasite's survival. On the other hand, total IgE could diminish the hazard of anaphylaxis and serve to protect the host (Hagan, 1993; Pritchard, 1993).

The results for patient 13, who was found seropositive for *Anisakis*, *Ascaris* and *Echinococcus*, are of particular interest. This patient showed a high serum concentration of *Anisakis*-specific IgE (with a CAP score of 5) and had a positive prick test. These results could indicate sensitization by *Anisakis*, with possible cross-reaction with *Ascaris* and *Echinococcus* antigens.

In the present study, the serum concentrations of anti-*Anisakis* IgE seen at presentation appeared unaffected by months on a fish-free diet. Sakanari *et al.* (1988) similarly observed that, in a patient with surgically confirmed anisakiasis, concentrations of anti-*Anisakis* IgE remained stable for at least 6 months. They found that the sera from patients who had a endured several episodes of ascariasis showed high levels of (cross-)reactivity against *Anisakis* antigen (with concentrations of anti-*Anisakis* IgE greater than those of anti-*Ascaris* IgE). However, patients with confirmed anisakiasis but with no history of contact with *Ascaris* showed high concentrations of anti-*Anisakis* IgE and did not react with *Ascaris* antigens. According to Sakanari *et al.* (1988), epitopes common to *Ascaris* and *Anisakis* ought to be processed during *Anisakis* infestation but might then be boosted during *Ascaris* infection. Similar findings were reported when cross-reactions with *Toxocara* were studied (Desowitz *et al.*, 1985). Patients diagnosed with anisakiasis showed high concentrations of anti-*Anisakis* IgE (as determined by RAST), while concentrations of anti-*Toxocara* antibody remained undetectable. In contrast, asthmatic patients, sensitized by *Toxocara*, gave positive RAST scores against both *Anisakis* and *Toxocara*. When serum from a patient with visceral larva migrans was studied, concentrations of IgE against *Anisakis* were found to be higher than those against *Toxocara* (Desowitz *et al.*, 1985).

The aim of a future study is to determine if the relatively stable concentrations of *Anisakis*-specific IgE observed in the present study are indicative of repeated sensitization by exposure to *Anisakis* or if, on the contrary, they have no predictive value.

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