

TIME COURSE OF TOTAL AND FOOD SPECIFIC IGE ANTIBODIES (RAST Fx5®) IN THE DEVELOPING ALLERGIC CHILD

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SUMMARY:

Aim: To analyze the evolution of total and food specific IgE (Rast Fx5®) titers in the course of food allergy in children.

Patients & methods: 925 children, mean age 8.9 ± 9.1 years (15 days-18 years), 455 girls and 470 boys, investigated for food allergy in year 1997, underwent serum samples assay for total IgE and of Rast Fx5®, Cap System, Pharmacia & Upjohn Diagnostics AB®, Uppsala, Sweden, a mix test detecting specific IgE to 6 major food allergens in children, cow's milk proteins (CMP), egg white, peanut, soy, wheat and fish and analyzed as a function of reference values by age.

Results: Total IgE titers increased significantly with age, $r=0.22$, $p=0.0001$. The percentage of children with a total IgE level above normal range first increased with age, from 10.3 % at 0-6 months, reaching 38.2 % at 1-2 years and peaking at 56.9 % at 4-6 years, then leveled off, 48.1 % at 8-12 years (ns vs 4-6 years) and 41.3 % above 12 years (ns). Detectable Rast Fx5® increased in a parallel manner until age 4-6 years and then exhibited a progressive decrease from age 6-8 years, differing significantly from total IgE: 31.9% vs 52.9%, $p=0.01$ (6-8 years), 32.2% vs 48.1%, $p=0.03$ (8-12 years), 21.8% vs 41.3%, $p=0.05$ (> 12 years).

Conclusion: In these outpatient children investigated for food allergy, the increase with age of the percentage of high total IgE contrasted with the progressive decrease of Rast Fx5® from age 4-6 yr. This biological finding correlates timely with the clinical spontaneous decrease of food allergy in the developing child.

Key-words: Total IgE - Food specific antibodies (Rast Fx5®) - Food allergy - Children.

INTRODUCTION

Several studies have addressed the detection of total IgE in cord blood (1, 2) and in the developing child (3). Total IgE exhibit a gross relationship with the development over years of atopic diseases (2), which may vary since not all patients with elevated specific IgE to food exhibit clinical symptoms (4) and allergic symptoms, such as eczema may occur in the absence of raised total or specific IgE (5). Peripheral blood IgE raise significantly with age according to several descriptions (1, 3), which serve as a basis for detection of abnormal levels.

Total IgE actually represent the addition of all specific IgE developed against various allergens, the first to develop being directed against food (2, 4, 6, 7), those

directed against inhalants developing mostly from the third year of age (2). Despite the particular pattern of food allergy, which the child is able to outgrow most of the time (4,6,7), as opposed to allergy to inhalants (4,6), a limited number of studies has investigated the evolution of specific IgE against foods on a large scale (2, 8-10). Also, few data compared their evolution with that of total IgE. Rast Fx5® is a blend of 6 different foods most commonly found in the allergic child, recommended for detection of individual food specific IgE, and therefore used in several settings on a routine basis.

This study was not performed to address the real values of total IgE or specific IgE of Rast Fx5® in the course of food allergy, as compared to the gold standard (i.e. open, single-blinded, and or double-blind placebo controlled oral food challenges), whereas the purpose of this cross sectional study was to describe the evolution pattern of peripheral blood total IgE and of Rast Fx5®, taking advantage of a routine measurement in a food allergy pediatric outpatient clinic to carry out a prospective systematic evaluation, regardless of any information about whether their foods were being withheld from their diet or not.

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PATIENTS AND METHODS

Patients

A cross sectional analysis was carried out in a population of 925 children, mean age was $8,9 \pm 9,1$ years (15 days to 18 years), 455 girls and 470 boys assayed between January and December 1997 following referral to an outpatient clinic of food allergy. This population exhibited at least one symptom of allergy, the main symptom being atopic dermatitis (35%), digestive manifestations (26%), pulmonary and ENT manifestations (8%), anaphylactic shock (4%) and combined manifestations (27%).

Methods

All children underwent a systematic blood sample for measurement of RAST Fx5®, a well calibrated *in vitro* laboratory technique combining six of the most antigenic food allergens in children, cow's milk proteins (CMP), white egg, peanut, soy, wheat and fish, using the Cap System (Pharmacia & Upjohn Diagnostics AB®, Uppsala, Sweden). Sera were analyzed using the RAST immuno-fluoro-enzymatic method calibrated with reference to the World Health Organization standards for IgE. Its specificity was already assessed (11) and its use in children already reported (12,13).

Total IgE were obtained in 868/925 of the children. Reference data for total IgE levels within normal range at each period of age were drawn from two independent studies performed in 466 carefully selected healthy children (14,15). Values below normal upper limit are summarized as follows: umbilical cord $<0,9$ KU/l ; 1 to 6 months: 0-15 KU/l ; 6 to 12 months: 0-20 KU/l ; 1 to 2 years: 0-30 KU/l ; 2 to 4 years: 0-45 KU/l ; 4 to 6 years: 0-60 KU/l ; 6 to 8 years: 0-100 KU/l ; > 8 years: 0-150 KU/l. Reference data for RAST Fx5® were those of the manufacturer considering values $< 0,35$ KU/l as non detectable.

Results are given as percentage above normal range for total IgE, percentage of detectable levels for mixed Rast Fx5® and in absolute values for total IgE, mixed food specific IgE Rast Fx5®.

STATISTICAL ANALYSIS

All statistical tests were performed with the Statview program (Abacus®, CA, USA). Calculation of mean, SD, median and extremes were done for all quantitative parameters. A correlation study between diffe-

rent quantitative parameters was performed using simple regression curves. Differences between qualitative parameters were analyzed by the chi-square test. A p value of < 0.05 was accepted as statistically significant.

RESULTS

Total IgE

A simple regression curve shows that total IgE titers in this pediatric population rose significantly with age, $r 0,22$ p 0,0001, figure 1.

The percentage of children with a total IgE level above normal range (table I, p.259) first increased with age, from 10.3 % at 0-6 months, reaching 38.2 % at 1-2 years and peaking at 56.9 % at 4-6 years, then leveled off, 48.1 % at 8-12 years (ns vs 4-6 years) and 41.3 % above 12 years (ns).

Absolute values of total IgE are depicted according to age in figure 2. Total IgE increased significantly from an age period to another from age 0-6 mo to age 4-6 years ($p<0.05$) and then leveled off with a median close to

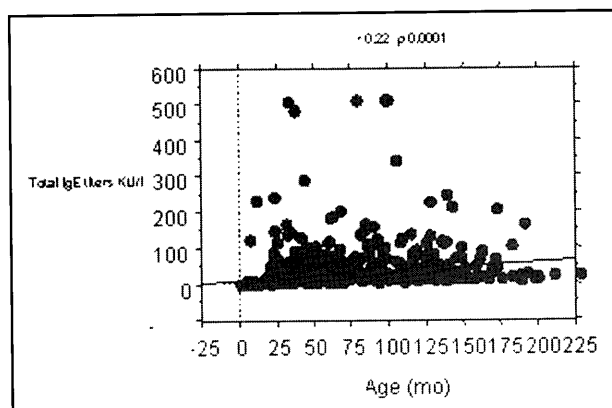


Figure 1: Correlation between total IgE titers and age in children, n=868.

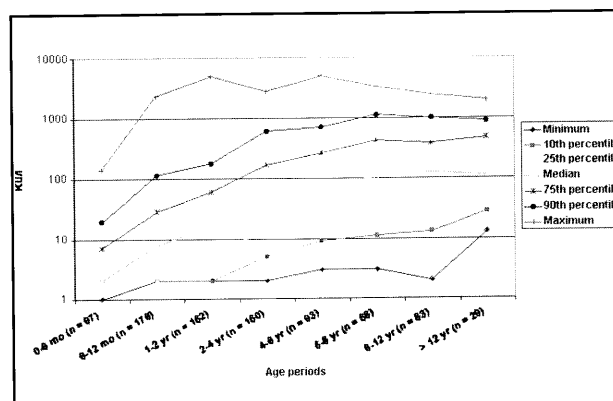


Figure 2: Total IgE titers according to age periods, n=868.

	0-6 months	6-12 months	1-2 years	2-4 years	4-6 years	6-8 years	8-12 years	> 12 years
<i>Values above normal range of Total IgE*£</i>								
	n = 97	n = 176	n = 162	n = 160	n = 93	n = 68	n = 83	n = 29
n (%)	10 (10,3)*£§#€λ	50 (28,4)*£α#€λ	62 (38,2)*π£§#	81 (50,6)*π£§α	53 (56,9)*π£	36 (52,9)*π£	40 (48,1)*π	12 (41,3)*
<i>Detectable values of food specific IgE titers (RSAT Fx5®)*£1</i>								
	n = 98	n = 181	n = 172	n = 175	n = 102	n = 72	n = 90	n = 32
n (%)	18 (18,3)*π£α§#€	54 (29,8)*£α§#€λ	72 (41,8)*π€	76 (43,4)*π€	47 (46)*π#€λ	23 (31,9)*§α	29 (32,2)*§α	7 (21,8)£α§

* p < 0,0001 Chi2 test, period 0-6 months vs other periods.

π p < 0,0001 Chi2 test, period 6-12 months vs other periods.

£ p < 0,05 Chi2 test, period 1-2 years vs other periods.

α p < 0,0001 Chi2 test, period 2-4 years vs other periods.

§ p < 0,0001 Chi2 test, period 4-6 years vs other periods.

p < 0,0001 Chi2 test, period 6-8 years vs other periods.

€ p < 0,0001 Chi2 test, period 8-12 years vs the other periods.

λ p < 0,0001 Chi2 test, period >12 years vs other periods.

*£ Values above normal range of Total IgE was defined according to Johansson SG et al, 1976, Bhalla RB et al, 1982).

*£1 Detectable values were defined upon the manufacturer considering as positive result values > 0,35 KU/l.

Table 1: Values above normal range for total IgE titers (n = 868) and detectable values of food specific IgE titers [RAST Fx5®] (n=922).

100KU/l. The highest total IgE level observed according to age periods started at 137 KU/l for 0-6 months, peaked above 5000 KU/l at 4-6 years, then progressively declined to 1967 KU/l in children above 12 years.

Food specific IgE (RAST Fx5®)

The percentage of children with a detectable Rast Fx5®, i.e. detectable mixed food specific IgE titers, first increased with age (table I), from 18.3 % at 0-6 months and peaking at 46 % at 4-6 years, then decreased significantly, 31.9 % at 6-8 years ($p < 0.05$ vs 0-6 months, 2-4 years and 4-6 years), 32.2 % at 8-12 years ($p < 0.05$ vs 0-6 months, 2-4 years and 4-6 years) and 21.8 % at >12 years ($p < 0.05$ vs 1-2 years, 2-4 years and 4-6 years).

Absolute Rast Fx5® values of detectable RAST Fx5 levels showed maximal levels exceeding 100 KU/l from 6 months to 6 years of age (figure 3). The 90th percentile level started from 28.5 KU/l at 0-6 months, peaked at 100 KU/l at 6-12 months, and then progressively declined to 4.44 KU/l in children above 12 years. A similar pattern was seen for the median value, from 1.1 KU/l at 0-6 months, to 3.49 KU/l at 6-12 months, and 0.86 KU/l in children above 12 years.

DISCUSSION

We provide a cross sectional analysis of the relationship between development of total IgE and of Rast Fx5® as an indicator of food specific IgE in a child

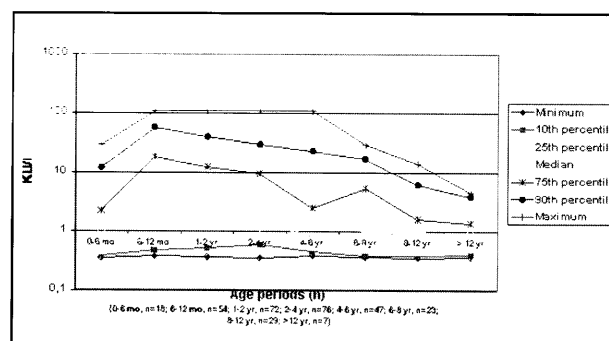


Figure 3: Detectable specific IgE (RAST fx5), n=326/922 (35.3%), distribution according to age. Detectable values were those above detection limit as indicated by the manufacturer ($p > 0,35$ KU/l).

population referred for evaluation of food allergy. An increase with time was seen both in the number of patients with IgE levels above reference values and in the median total IgE level, both parameters stabilizing from age 4-6 years. In the same cohort, the percentage of children with detectable Rast Fx5® -i.e. specific IgE directed against 6 major food allergens- peaked at age 4-6 years before a gradual decrease, in contrast with total IgE. Absolute levels of Rast Fx5® peaked earlier, between 6 and 12 months, also with a following decrease.

The kinetics of total IgE in the developing child have already been investigated, especially by Johansson SG et al (14) and Bhalla RB et al (15), whose studies are used as reference by the manufacturer of the IgE measurement system. Several authors confirmed the progressive increase in total IgE levels according to

age in children (1,3). For Lindberg RE and Arroyave C (1), serum IgE levels in 346 non-allergic and 301 allergic children indicated an increase of the geometric mean for non-allergic patients from 0.9 IU/ml at less than 1 year of age to 45.4 IU/ml at 13-16 years of age. *Berciano et al (16)* also showed that the geometric mean of serum IgE increases with age and higher mean values were found for children with a family history of allergy. The cut-off between normal and abnormal values therefore increases with age as further confirmed by Lindberg RE and Arroyave C (1), who calculated an increase from 10 IU/ml at less than 1 year to 100 IU/ml at 13 to 16 years of age. This cut-off value is difficult to delineate precisely as shown by Saarinen *et al* (3) who compared healthy, non-atopic children from birth to 5 years of age with children having latent, symptomatic, or severe atopic disease, and suggested that a zone of uncertainty, lying between the 95th and 97.5th percentiles, should replace the upper limit of normal. This may explain why for Backer *et al* (17), in a random sample of Danish children and adolescents, aged 7-16 years, normal IgE values were considered up to 330 kU/l.

The kinetics of food specific IgE in the developing child have also been addressed by several authors (2,4,6-10). IgE to foods are seldom detected in cord sera but several children will develop low titers already during the first months of life, often without obvious preceding contact with the antigen, in agreement with the role of intrauterine sensitization, already largely acknowledged (18). Hattevig G *et al* (4) showed in a cohort of 86 healthy babies, that specific IgE against egg, cow's milk and soy were detectable at 3, 8, 25 and 48 months in 9, 23, 6 and 2 children respectively. Sigurs N *et al* (2) also detected IgE directed against at least 1 allergen in 135/324 (42%) children followed from birth and directed to egg white in 46 (14%) children at age ≤ 2 years. A recent prospective Estonian cohort of children with a low prevalence of atopy (8,10) exhibited 13, 23, 36 and 36% children with IgE antibodies against food allergens at 0.5, 1, 2 and 5 years (9).

The origin of the IgE rise has to be analyzed both in regard to the ability of the atopic organism to mount an IgE reactivity and in terms of specific triggering induced by allergens. The mechanism by which orally ingested allergens elicit an IgE response remains unclear especially since there are few animal models available for investigation of this response. Oral administration of ovalbumin to an ovalbumin-specific T-cell receptor transgenic mice increased the levels of both antigen-specific IgE and total IgE in the sera (19). The present data indicate that until age 4-6 years increase in food specific IgE response grossly paralleled that of total IgE but concordance analysis also

shows that total IgE may raise when food specific ones do not and that the reverse is true; specific subsets of allergic patient may therefore proceed from a predominantly ability to get sensitized to several foods, whereas in others, elevated specific IgE might participate in a global process affecting all IgE production.

Following the increase in food specific IgE in the first years of life, a down regulation occurs both in the number of positive results and in the plasma levels achieved. This down regulation of food specific IgE has already been suggested by different studies. In the cohort of Hattevig G *et al* (6), of the 8 children with elevated IgE to egg white during infancy, only one still had increased concentrations at 7 years of age and no child developed an elevated IgE level to egg white or milk after the first year of life. The follow-up by Sigurs N *et al* (2) of patients until 12 years of age showed a peak prevalence of IgE to cow's milk and egg white at age 8 months. The specific contribution of the present data may be synthesized as follows. First, the highest percentage of positive Rast Fx5[®] results was observed in the 4-6 year of age period and was followed by a gradual decrease with age, contrasting with persistent high percentages of elevated total IgE. Second, the highest Rast Fx5[®] levels seems to be achieved in the 6-12 months period, even though the number of children with detectable Rast Fx5[®] values increases up to the 4-6 year period. Third, when considered individually, the contribution of food specific IgE to Rast Fx5[®] positivity varies with time, egg representing the first sensitizing antigen, as previously described (4), milk generating a similar pattern, with a down regulation observable from the 2-4 years period and peanut generating a delayed sensitization with no indication of a down regulation. This pattern of egg specific IgE is, in addition, similar to that described for IgG1 and IgG3 subclass antibodies to ovalbumin which peak at 18 months and then decline up to age 8 years (20).

When food specific IgE decrease, total IgE level remains stable. This probably illustrates the role played beyond the first years of life by specific IgE to inhalants, which develop while food specific ones progressively decrease. In the children followed by Sigurs N *et al* (2) IgE to inhalant allergens appeared in approximately 33% of children during the first 4 years of life and in 50% of them before 12 to 15 years. Interestingly, in more than half of children with specific IgE to egg will develop IgE to inhalants within the next 2 years (7). It may be considered, despite the absence of IgE to inhalants in that study, that the child maintains high IgE levels through a response to inhalants despite a decrease of food specific antibodies.

The significance of this kinetics has to be analyzed cautiously. Measurement of IgE as the only screening for allergic disease is unreliable, as the predictive value of elevated IgE in population samples was found to be 50% (17) even though in this cohort, the rate of positivity of total IgE measurements indicates values close to previously described cohorts of allergic children (2). In addition, a transient IgE response to foods may be seen even in some children who show no signs of clinical allergy. Thus, IgE antibodies to milk and egg white appear frequently in both atopic and non-atopic infants, but high concentrations are found mainly in infants with present or future atopy. In the study by Hattevig *et al* (6), 26% of normal-risk infants developed IgE antibodies to egg white or cow's milk during the first 18 months of life compared with 53% of the high-risk children.

However, food specific IgE may be responsible for at least a part of food adverse reactions and approximately one third of children with refractory, moderate-severe atopic dermatitis have IgE-mediated clinical reactivity to food proteins (21). Several food may be involved including, apart from those largely acknowledged at this age, peanut, egg and milk, some others such as wheat (22). In that respect, it may be stressed that food specific IgE titers decline with age in good agreement with the decreasing rate of clinical manifestations of allergy with age, as already shown by individual longitudinal follow-ups (23). In patients with atopic dermatitis and food hypersensitivity who were adhering to an elimination diet, 25% of patients lost all signs of clinical food hypersensitivity after 1 year (24). Loss rate of food hypersensitivity varied among foods; after 1 year, there was a 26% loss of symptomatic food allergy to five major allergens (egg, milk, soy, wheat, and peanut) compared with a 66% loss rate to other food allergens (24).

In summary, this cross sectional analysis provides an approach to the kinetics of total and food specific IgE to food in allergic children, indicates that 1/ the number of detectable food specific IgE results increase until the age of 4-6 years and thereafter decrease while total IgE keep the same level of abnormal values 2/ Rast Fx5® values seem to peak early, at age 6-12 months, with a gradual decline thereafter.

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