

Possible Modes of Allergen-Specific Sensitization and Boosting in an Atopic Child

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Key Words

Egg allergy · IgE · Sensitization · Food allergy

Abstract

Several studies document that allergen-specific IgE levels are boosted by allergen contact via the respiratory tract in allergic patients. Only few data are available on whether other routes of allergen contact have an influence on systemic IgE responses. We report the case of a boy who developed egg allergy after heavy consumption of eggs by the mother during pregnancy and breast feeding. In contrast to other children who outgrow egg allergy during the first years of life, the boy experienced further dramatic increases in hen egg-specific IgE antibodies after prolonged consumption of ostrich eggs containing cross-reactive allergens. IgE antibodies to most of the important respiratory allergens remained either low or not detectable. The dramatic increases in hen egg-specific IgE antibody levels after oral intake of allergens demonstrate that systemic IgE responses in allergic patients can be strongly boosted by allergen contact via routes other than the respiratory tract.

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Introduction

Hen's egg and cow's milk proteins are the most common elicitors of food allergies in early childhood [1]. Allergic children frequently outgrow most food allergies during the first 5 years of life whereas sensitization to respiratory allergen sources develops continuously thereafter [1]. IgE antibody responses to respiratory allergens can be detected shortly after birth in 10% of atopic children in response to allergen contact and are strongly boosted by repeated respiratory allergen contact [2]. This strong boost of systemic IgE antibody production is particularly well documented for seasonal respiratory allergens [3].

In the case of food allergens there is evidence for a pre-natal in utero priming which may pave the way for the subsequent development of food allergies [4]. Whether postnatal oral allergen intake can aggravate food allergy [5] or induce tolerance against food allergens is a matter of controversy. On the one hand, most children outgrow food allergy and several experimental animal models demonstrate that allergen contact via the gastrointestinal tract induces immunological tolerance [1, 6–8]. On the other hand, it has been reported in a murine model that low-dose systemic priming followed by intragastric challenge leads to allergic sensitization [9].

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We have monitored the development of IgE antibody levels to food and respiratory allergens in a boy during the first 5 years of life. The onset and course of egg allergy in this boy indicate that prenatal priming and allergen contact via the gastrointestinal tract were events which were associated with dramatic increases in allergen-specific systemic IgE antibody levels.

Material and Methods

Case

We have quantified IgE levels specific for egg and milk allergens during the first 5 years of life in a boy suffering from egg and milk allergy. The boy's mother, who is allergic to insect venoms, reported massive consumption of eggs (at least 5 eggs each day) during pregnancy and lactation. From the age of 3.5 months, when baby food (Aptamil 1, Milupa, Puch/Salzburg, Austria; Hipp 1, Hipp, Pfaffenhofen/Ilm, Germany) was introduced in addition to breastfeeding, the child suffered from repeated episodes of diarrhea. The mother was advised to exclusively breastfeed the child, but the diarrhea persisted. At the age of 6 months, egg-specific IgE levels > 30 kUA/l were measured, indicating a clinically relevant sensitization to egg proteins [10]. Furthermore, milk-specific IgE antibody levels were elevated (6.5 kUA/l). The possible clinical relevance of egg- and milk-specific IgE levels was also underlined by positive skin prick test reactivity. The mother was advised to strictly avoid egg and milk consumption during breastfeeding and subsequently the child was set on an egg- and milk protein-free diet, employing various formulas of hypoallergenic baby food (Som, Milupa; Profylac, Arla Foods, Viby, Denmark; Alfaré, Nestlé, Vevey, Switzerland), but the child further suffered from diarrhea and experienced episodes of urticaria. From the age of 7.5 months until the age of 2.5 years the child was fed with Pregomin (Milupa) and vegetable purées (carrots, potatoes).

After administration of vaccines (Central European encephalitis, polio Salk, diphtheria, tetanus, pertussis, haemophilus) at the age of 21 months (May 1998), the child exhibited local reactions and fever followed by severe symptoms of bronchial obstruction. From June 1998 to February 2001 the episodes of diarrhea and severe bronchial obstruction after food intake persisted, requiring administration of antihistamines and steroids. A retrospective anamnesis revealed that the child was fed ostrich eggs since May 2000.

The possible effects of allergic symptoms on the physical development of the child are indicated by the fact that the height and weight development decreased from 50% in the first 6 months, to 10% at the age of 1 year (August 97), and to 3% at the age of 4.5 years (February 2001).

Determination of Total and Allergen-Specific IgE/IgG

Serum samples from February 1997 and April 1998 had been analyzed for the presence of total and egg white-specific IgE by CAP FEIA determinations (Pharmacia Diagnostics, Uppsala, Sweden). Further serum samples were collected from September 1999 to February 2001 on the occasion of hospital visits. The latter serum samples were analyzed for egg-specific IgG antibodies as well as for IgE antibodies specific for a panel of respiratory (cat and dog dander, timothy grass, ragweed, mugwort, birch and hazel pollen, latex, mite, mould mix) and food allergens (egg white, egg yolk, ovalbumin, ovo-

mucoïd, conalbumin, cow's milk, α -lactalbumin, β -lactoglobulin, casein, meat, fish, wheat, soybean, carrot, nutmix) by CAP FEIA measurements (Pharmacia, Uppsala, Sweden).

Preparation of Hen and Ostrich Egg Extracts, SDS-PAGE and IgE Immunoblotting

One gram of hen's egg white and 1 g of ostrich egg white were boiled each in 20 ml SDS sample buffer [11]. Approximately 100 μ g/cm preparative gel of each protein extract were separated in 12% SDS-PAGE. SDS-PAGE, transfer and immunoblotting were performed as described [12]. Patient's serum was diluted 1:10 (ostrich) or 1:50 (hen) in PBS containing 0.1% v/v Tween 20 and exposed to nitrocellulose-blotted egg white extracts. Blots were washed and bound IgE antibodies were detected with ^{125} I-labeled anti-human IgE antibodies (Pharmacia) as described [13].

Results

Quantification of Total and Allergen-Specific IgE Levels

When food allergy was first diagnosed in the child in February 1997, total IgE was 190 kUA/l and specific IgE levels were as follows: cow's milk: 6.5 kUA/l; egg white: 30.2 kUA/l (fig. 1). In a serum sample which was obtained 16 months after the child had been vaccinated, on the occasion of a hospitalization (September 1999) due to an episode of acute pulmonary obstruction, egg white-specific serum IgE levels had strongly increased to 1,072 kUA/l (fig. 1). In addition, a positive skin prick test to animal dander extracts (dog, cat) was observed, whereas no IgE responses to other common inhalant allergen extracts (pollens, mites, moulds) could be detected. In serum samples collected during the next 6 months, egg-specific IgE had declined to 576 kUA/l (October 1999) and remained practically unaltered from January 2000 (483 kUA/l) to March 2000 (555 kUA/l). However, dramatic increases in egg-specific IgE levels were found in serum samples obtained in November 2000 (1,342 kUA/l) and in February 2001 (2,020 kUA/l, fig. 1). A retrospective anamnesis revealed that the mother had fed ostrich eggs to the child during the latter period (i.e., since May 2000). The analysis of total and specific IgE levels for egg, milk and respiratory allergens between September 1999 and February 2001 indicated that most of the total IgE was directed against egg allergens (fig. 1). While egg allergen-specific IgE levels increased strongly after vaccination and ostrich egg consumption, milk allergen-specific IgE levels remained almost unaltered during this period. The variations of egg-specific IgG antibody levels followed the levels of allergen-specific IgE, but were much less pronounced (table 1).

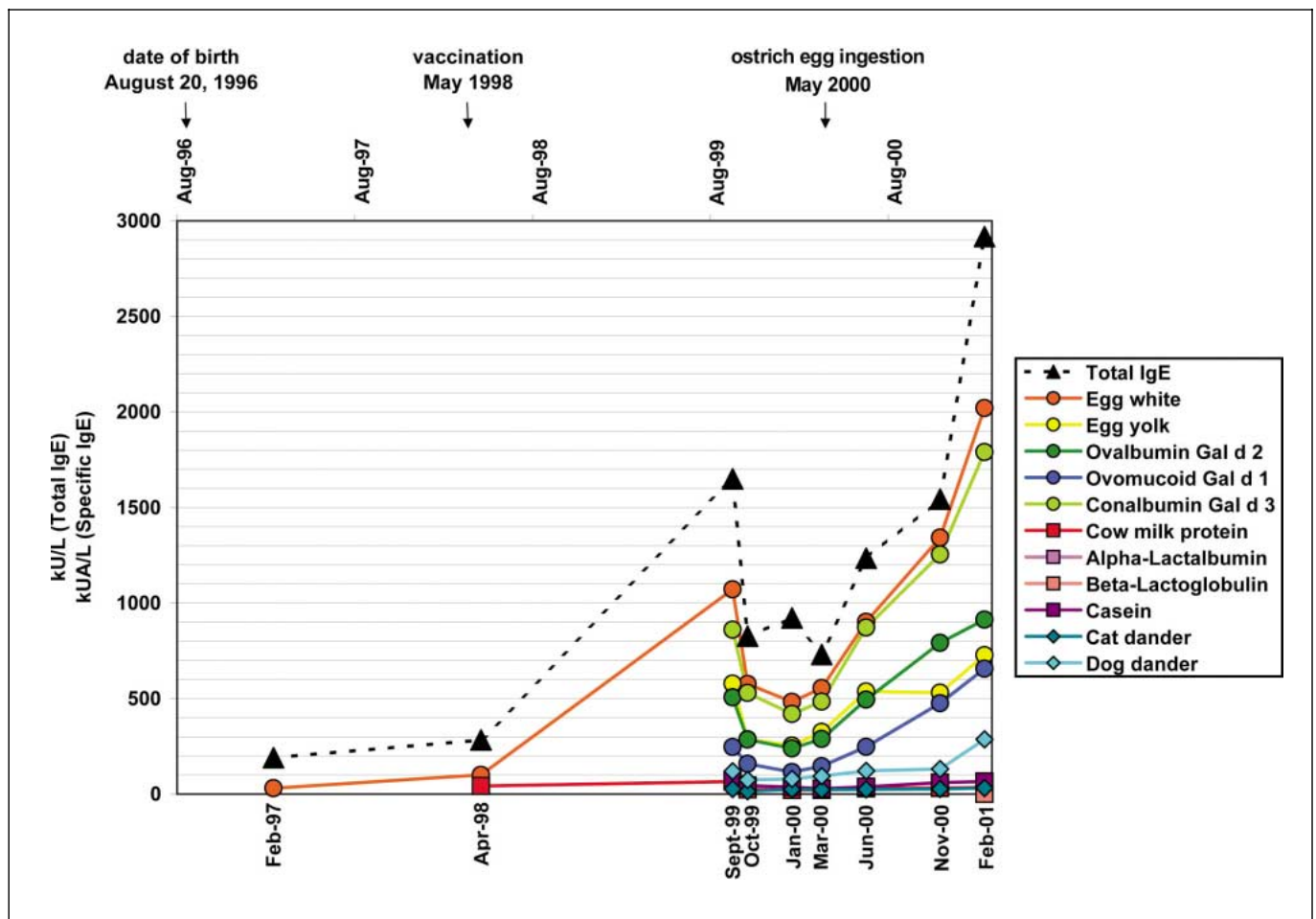


Fig. 1. Total and specific serum IgE levels during the first five years of life. Total IgE (kU/L) and specific serum IgE levels (kUA/L) (y-axis) were determined at the time points indicated at the bottom of the x-axis using CAP FEIA measurements (Pharmacia). Date of birth, years of life as well as dates of vaccination and dietary egg uptake are indicated on top.

With the exception of cat and dog dander allergens, no other common respiratory allergen sources (timothy grass pollen, birch pollen, mites, moulds, latex) induced significant IgE antibody levels during this period. Sensitization to meat (pork: 3.75 kUA/L; beef: 3 kUA/L; chicken: 7.22 kUA/L), hazelnut (21 kUA/L) and apple (3 kUA/L) could be detected by IgE measurements. No other significant sensitizations could be found.

Qualitative Analysis of IgE Antibodies Specific for Hen and Ostrich Egg Allergens by Immunoblotting

When nitrocellulose-blotted egg white protein extracts from hen and ostrich were exposed to the child's serum, several IgE-reactive components could be identified. A

Table 1. Egg white- and egg yolk-specific IgG levels (mgA/l)

	Egg white-specific IgG, mgA/l	Egg yolk-specific IgG, mgA/l
September 1999	19.7	15.3
October 1999	17.0	11.4
January 2000	12.8	8.8
March 2000	n.d.	n.d.
June 2000	n.d.	n.d.
November 2000	14.8	11.8
February 2001	16.7	13.5

Egg white- and egg yolk-specific IgG levels (mgA/l) were determined using CAP FEIA measurements at different time points. n.d. = Not determined.

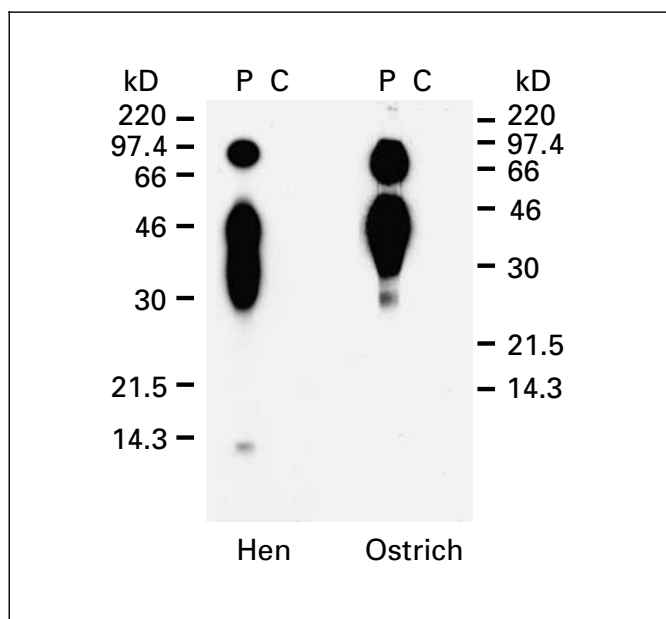


Fig. 2. Hen's and ostrich egg white proteins were separated by SDS-PAGE and blotted onto nitrocellulose. Lanes P show the IgE binding pattern to egg white proteins from hen and ostrich. Serum from a nonallergic individual was used as control (lanes C).

similar pattern of IgE-reactive bands (88, 45, 28 kD), presumably representing conalbumin, ovalbumin, ovomucoid, were detected in hen and ostrich egg white extracts (fig. 2). A 14 kD IgE-reactive band possibly representing lysozyme, was only detected in hen egg white extracts (fig. 2).

Discussion

The case of egg allergy reported here seems remarkable for the following reasons: the disease course and allergen profile in the boy is different from the natural course of sensitization to food and inhalant allergens [1]. Whereas allergies to cow's milk and egg reportedly decrease during the first 5–6 years and sensitization to respiratory allergens increases in this period, we observed a continuous aggravation of egg allergy in the boy. The potential sensitization and boosting mechanisms behind this course are even more intriguing. In the case of respiratory allergies, sensitization occurs after birth and is triggered by repeated contact with respiratory allergens [2]. In our case, it is possible that prenatal allergen uptake and allergen uptake during breastfeeding contributed to the priming of the allergic immune response.

Although not proven, it is possible that the administration of vaccines produced using chicken embryo fibroblasts may have contributed to the induction of egg-specific IgE responses because milk allergen-specific IgE in contrast to egg-specific IgE remained low after vaccination). In this context, it has been mentioned that egg allergy may represent a potential hazard regarding vaccination with vaccines containing egg protein [14]. On the other hand, it has been demonstrated that vaccines produced using chicken embryo fibroblasts contain egg proteins, but can be safely administered to egg-allergic children [15, 16].

However, the most intriguing aspect of this study seems to be the fact that dietary intake of allergens may have boosted systemic IgE responses in this child. This assumption is supported by the following observations: first, egg-specific IgE declined and remained stable when eggs were omitted from the diet between September 1999 and March 2000. Second, a tremendous increase in egg-specific IgE was noted when ostrich eggs containing cross-reactive IgE epitopes were fed starting in May 2000 (fig. 1). We consider the possibility that high-dose allergen exposure via the gastrointestinal tract may have induced strong systemic IgE production in this child remarkable for the following reasons:

First, we are not aware of any report describing systemic rises of allergen-specific IgE antibodies following oral allergen intake, although respiratory allergen contact is known to boost allergen-specific serum IgE responses [3]. It may also be assumed that oral/mucosal administration of large amounts of allergens or their proteolytic fragments/peptides via the gastrointestinal tract will induce tolerance rather than immunity [17].

In conclusion, our case provides evidence for prenatal priming to allergens, sensitization/boosting during breastfeeding and dietary intake of allergens, and, although unproven, for possible boosting by injection of vaccines produced using chicken embryo fibroblasts.

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