

Total IgE and Specific IgG Levels to β -Lactoglobulin and Ovalbumin and Allergy Outcome in Small for Gestational Age Infants: A Pilot Study

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ABSTRACT

Total IgE and specific IgG levels to β -lactoglobulin and ovalbumin and allergy development were investigated in 22 small for gestational age (SGA) infants monitored for 12 months. A high incidence of allergy was observed at 12 months corrected age relative to published reports on appropriate for gestational age infants. This was particularly noticeable in infants from a nonatopic background, where 56% (5/9) were allergic, as well as in infants with a family history of atopy, where 85% (11/13) were diagnosed as allergic at 12 months corrected age. There was a nonsignificant (NS) trend for total serum IgE levels to increase with age in infants from both atopic and nonatopic backgrounds. Levels of β -lactoglobulin IgG peaked at 4 months corrected age in both groups and had decreased by 12 months corrected age, reflecting the induction of tolerance (median and range, 4 months corrected age vs. 12 months corrected age; atopic: 904 (8–3437) $\mu\text{g/ml}$ vs. 449 (53–3200) $\mu\text{g/ml}$, $p < 0.02$; nonatopic: 836 (39–1473) $\mu\text{g/ml}$ vs. 503 (85–1914) $\mu\text{g/ml}$, $p = \text{NS}$). Ovalbumin IgG antibody levels decreased between 40 weeks gestational age and 4 months corrected age (atopic: 66 (9–382) $\mu\text{g/ml}$ vs. 33 (6–599) $\mu\text{g/ml}$, $p = \text{NS}$; nonatopic: 40 (8–1026) $\mu\text{g/ml}$ vs. 16 (7–62) $\mu\text{g/ml}$, $p < 0.05$) before reaching their highest level at 12 months corrected age [atopic: 551 (17–3147) $\mu\text{g/ml}$, both 40 weeks gestational age and 4 months corrected age vs. 12 months corrected age, $p < 0.005$; nonatopic: 286 (13–1791) $\mu\text{g/ml}$, $p = \text{NS}$]. These changes reflect the degradation of maternal IgG in the infant and lack of egg in the early diet, followed by the introduction of egg products during weaning. This finding of a high allergy incidence in SGA infants should be further investigated. (Pediatr Asthma Allergy Immunol 1999; 13[4]:169-175.)

INTRODUCTION

APPROXIMATELY 5% OF INFANTS are born small for gestational age (SGA), although this figure varies from 3% to 13% depending on the cutoff used in defining the condition.⁽¹⁾ These infants have increased perinatal mortality and morbidity relative to their appropriate for gestational age (AGA) counterparts.⁽²⁾

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However, because of various *in utero* causes such as placental insufficiency, infants born SGA are a heterogeneous group. Some infants included in the definition will be SGA because of constitutional causes and may not be adversely affected. Others will have growth retardation caused by placental insufficiency *in utero*. The detriment to health not only is associated with the perinatal period but also has long-term consequences, with infants born SGA having been shown to be at greater risk than AGA infants of conditions such as non-insulin-dependent diabetes mellitus, coronary heart disease, and stroke.⁽³⁾

Little is known about the immunologic status of SGA infants. The full-term neonatal immune system is immature at the cellular level.⁽⁴⁾ Deprivation of nutrients in fetal life because of placental insufficiency may result in slow growth and low transfer of maternal IgG, resulting in the delay of the development of their immunological system. A study, in India, investigating the immune status of 25 full-term SGA infants⁽⁵⁾ found a low lymphocyte percentage, altered ratio of CD4+ and CD8+ subset of T-cells, and lower IgG levels relative to their AGA counterparts. The authors suggested that these observations might explain the increased rates of infection seen in SGA infants compared with AGA infants. The immature immune system may also predispose SGA infants to a greater risk of allergy development.

The prevalence of atopic disorders such as asthma, allergic rhinitis, and atopic dermatitis in childhood is increasing,⁽⁶⁾ with studies showing that 47.6% of school-age children have some form of atopic disease in their lifetime.⁽⁷⁾ In young children, the main symptoms are atopic dermatitis and food allergy.⁽⁸⁾ These have been shown to progress to the potentially more serious allergic respiratory conditions in many of those affected,⁽⁹⁾ while the initial symptoms alleviate. Raised levels of total serum IgE are common in full-term infants with allergy.⁽¹⁰⁾ A tendency toward increased levels of specific IgG antibodies to food has been observed in atopic subjects compared with nonatopic controls.⁽¹¹⁾ However, their presence has also been demonstrated in serum from healthy children.⁽¹²⁾

This study was undertaken to obtain baseline information on total IgE and specific IgG levels to β -lactoglobulin and ovalbumin and allergy development in SGA infants, to be used in future studies to assess the impact of intervention measures. This article describes the results from a pilot study in which serum total IgE and specific IgG to two common food proteins, β -lactoglobulin and ovalbumin, were measured in SGA infants from both atopic and nonatopic backgrounds at intervals until 12 months corrected age, when the infants were assessed for development of clinical allergic disease.

METHODS

Approval for the study was obtained from appropriate local ethical committees. Data were collected by two trained research midwives.

Subject recruitment

For this prospective study, 22 SGA infants defined as birth weight $\leq$ third percentile for gestational age⁽¹³⁾ were recruited at delivery from three local hospitals. Informed written consent was obtained from the parents.

Data collection

A structured interview was conducted with the mother at birth, 40 weeks gestational age of the infant, and 4 and 12 months after this date (4 and 12 months corrected age, respectively) to ascertain patterns of milk and solid food feeding (weaning). Infants fed their first solid food before 16 weeks from birth were classified as early weaners. Infants fed their first solid food at 16 weeks or later were classified as late weaners. Measurements of weight, length, and head circumference were also made at this time. All infants were fed a combination of breast and formula milk before the introduction of solid food.

Parental allergy assessment

The atopic background of the infants was assessed using a three-point ranking system consisting of: (1) a family allergy history questionnaire (developed in the University of Surrey), (2) elevated parental total

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IgE antibody levels; and (3) positive parental allergy skin tests. A parent was considered atopic if two of these three criteria were positive.

As a part of the family allergy history questionnaire, both parents were asked if they had any symptoms of allergy, e.g., asthma, eczema, hay fever, or adverse reactions to foods; any known cause of the symptoms; whether the symptoms had been diagnosed by a physician; whether any medication was taken to alleviate the symptoms; and whether any other family members (parents, siblings, children) also had any allergies.

Parental total IgE antibody levels were considered elevated if the IgE was >80 kU/L.

Parental skin-prick allergy testing was performed using ALK Lancet to nine environmental and food allergens [three trees (hazel, alder, silver birch), six grasses (False oat, Cocksfoot, Meadow fescue, Rye, Timothy and June grasses), cat, dog, *Alternaria alternata*, *Cladosporium herbarum*, *Dermatophagoides pteronyssinus*, egg, and cow's milk (Soluprick, ALK, Copenhagen)]. A wheal greater than or equal to the histamine dihydrochloride (10 mg/ml) control response was regarded as positive when the saline control gave a negative result.

Blood samples and biochemical analysis

Blood samples were taken from the infants at 40 weeks gestational age and at 4 and 12 months corrected age. The first two samples were obtained by the heel prick technique, and the last sample was taken by venipuncture from the back of the hand. Blood samples were also obtained by venipuncture from the parents. The samples were allowed to clot, and the serum was removed and stored frozen at -20°C until analysis.

IgG antibodies to ovalbumin (OVA) and β -lactoglobulin (β LG) were measured using in-house indirect enzyme-linked immunoassay (ELISA).⁽¹⁴⁾ Total serum IgE antibody measurements were made using the Pharmacia UniCAP system (limit of detection 0.35 kU/L for infant samples and 2 kU/L for adult samples) by the Department of Immunology, Royal Surrey County Hospital, Guildford, UK.

Allergy assessment of the infants

Infants were assessed by a pediatrician, unaware of the atopic history of the infant, at 12 months corrected age for signs of allergy over the previous year by physical examination and structured parental interview (personal communication, J.O. Warner, Southampton). This included assessment of any recurrent skin, chest, nasal, eye, and gastrointestinal symptoms. Atopic dermatitis was defined as dryness, erythema, itching, or excoriation lasting >30 days in 1 year. The degree of severity was assessed using the SCORAD index.⁽¹⁵⁾ Respiratory illness was defined as a persistent recurrent nocturnal cough disturbing sleep in >3 consecutive nights or a wheeze of any duration. Nasal symptoms were defined as persistent recurrent sneezing, runny nose, blocked nose, any of two of these three symptoms lasting >2 weeks in an episode. Eye symptoms were defined as watery, itchiness, redness, or swollen eyelids for >48 h in any one episode, not associated with infection. Gastrointestinal illness was defined as >48 h of vomiting or diarrhea (not associated with infection) or of swelling of the lips. An infant was classified as being allergic if he or she had any of these disorders.

Statistical analysis

Statistical analysis was undertaken using SPSS for Windows version 7. The distribution of the immunological data was nonparametric. Comparisons between groups of infants from atopic and nonatopic backgrounds were undertaken using the Mann-Whitney U test. Comparisons within the groups were made using the Wilcoxon signed ranks test. Results were taken as significant if $p < 0.05$. Data were expressed as median and range.

RESULTS

Subject characteristics

Thirteen infants had a positive family history of allergy and were therefore classified as atopic, and nine were nonatopic. In the atopic group, six infants had both parents atopic, three infants had mothers with

TABLE 1. SUBJECT CHARACTERISTICS OF SGA INFANT GROUP (MEDIAN [RANGE])

	<i>Atopic background</i> (n = 13)	<i>Nonatopic background</i> (n = 9)
Gender (M:F)	5:8	5:4
Gestational age at birth (wk)	38 (29–40)	37 (31–41)
Birth weight (kg)	2.35 (0.92–2.88)	2.19 (1.08–2.61)
Weaning age from birth (wk)	16 (13–20)	17 (10–22)
No. (%) of early weaners	8 (62%)	4 (44%)
Weaning weight (kg)	5.13 (4.07–6.43)	4.83 (4.54–6.32)
No. (%) of infants allergic at 12-month assessment	11 (85%)	5 (56%)

atopic backgrounds, and four infants had fathers with atopic backgrounds. In this group, 80% of the mothers and 56% of the fathers had positive skin prick test results. In the nonatopic background group, only 22% of the fathers and none of the mothers had positive skin prick test results. Table 1 shows the subject characteristics, weaning patterns, and allergy status of the infants grouped according to their family history of atopy.

There were no significant differences between the infants from atopic or nonatopic backgrounds for gender, gestational age at birth, birth weight, weaning age from birth, percentage of infants weaned early, or weight at weaning. Fifty-six percent (5/9) of infants from a nonatopic background and 85% (11/13) of infants from an atopic background were diagnosed as allergic at 12 months corrected age, but this difference was not statistically significant. The most predominant allergic manifestation was eczema. The majority of infants who were diagnosed as allergic at 12 months corrected age displayed this condition (77% [10] of infants from an atopic background and 44% [4] from a nonatopic background).

Serum total IgE

Table 2 shows the results of serum total IgE measurements for infants from atopic and nonatopic backgrounds at 4 and 12 months corrected age. There was insufficient sample to measure total serum IgE at the 40 weeks gestational age time point. Total IgE levels increased with time in both groups, but there were no significant differences in total IgE levels between infants from atopic or nonatopic backgrounds at either of the time points.

Specific IgG to β -lactoglobulin and ovalbumin

Table 3 shows the specific IgG antibody levels to β LG and OVA in infants from atopic and nonatopic backgrounds at 40 weeks gestational age, and 4 and 12 months corrected age. Results with the same superscript symbols were significantly different from each other.

IgG antibodies to β LG increased between 40 weeks gestational age and 4 months corrected age before returning to a level between these two values by 12 months corrected age in both atopic and nonatopic groups. In the infants with an atopic background, the drop between 4 and 12 months corrected age was statistically significant ($p < 0.02$). There were no statistically significant differences in β LG IgG antibody levels between infants from atopic and nonatopic backgrounds at any time point.

TABLE 2. TOTAL IgE LEVELS (kU/L) IN INFANTS FROM ATOPIC AND NONATOPIC BACKGROUNDS (MEDIAN AND RANGE)

	<i>Atopic background</i>	<i>Nonatopic background</i>
4 months corrected age	3.75 (0.69–23.80)	5.1 (1.05–39.60)
12 month corrected age	5.02 (0.56–59.10)	10.40 (1.05–39.70)

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TABLE 3. SPECIFIC IgG LEVELS TO β -LACTOGLOBULIN AND OVALBUMIN (μ G/ML) IN INFANTS FROM ATOPIC AND NONATOPIC BACKGROUNDS (MEDIAN AND RANGE)

	<i>β-Lactoglobulin</i>		<i>Ovalbumin</i>	
	<i>Atopic</i>	<i>Nonatopic</i>	<i>Atopic</i>	<i>Nonatopic</i>
40 weeks gestational age	72 (11–673)	119 (46–617)	66 (9–382) ^b	40 (8–1026) ^d
4 months corrected age	904 (8–3437) ^a	836 (39–1473)	33 (6–599) ^c	16 (7–62) ^d
12 months corrected age	449 (53–3200) ^a	503 (85–1914)	551 (17–3147) ^{b,c}	286 (13–1791)

^a $p < 0.02$; ^{b,c} $p < 0.005$; ^d $p < 0.05$.

By 4 months corrected age, IgG antibodies to OVA had dropped to approximately half those of the 40 weeks gestational age level, before peaking at 12 months corrected age. These patterns applied to infants from both atopic and nonatopic backgrounds. In the atopic infants, the increases between 40 weeks gestational age and 12 months corrected age and between 4 and 12 months corrected age were both statistically significant ($p < 0.005$). In the nonatopic group, the decrease between 40 weeks gestational age and 4 months corrected age was statistically significant ($p < 0.05$). OVA IgG antibody levels were higher in atopic than nonatopic infants at all time points, but the differences were not statistically significant.

DISCUSSION

The incidence of allergic disease in this group of SGA infants appeared to be very high. This has not been previously reported, to our knowledge. Eighty-five percent of infants from an atopic background showed signs of allergic disease. Figures quoted in the literature for AGA infants range from 25% to 50% depending on whether one or both parents are affected.⁽¹⁶⁾ What is perhaps more interesting is that 56% of SGA infants without a family history of atopy were also allergic, compared with approximately 10% to 15% in the general population.⁽¹⁶⁾ Sensitization to allergens can occur as early as 22 weeks gestation *in utero*.⁽¹⁷⁾ Early events in the development of the SGA condition may accentuate this sensitization, causing the high incidence of allergic disease observed in these SGA infants.

Median total IgE levels appeared to increase with time in infants from both atopic and nonatopic backgrounds, although the differences were not significant. This reflects their increasing exposure to allergens with age. Levels of total IgE were similar in infants from both backgrounds and also between infants diagnosed as allergic or nonallergic at 12 months corrected age. This suggests that measurement of total IgE may not be sensitive enough to discriminate between subgroups within this population. Using stepwise logistic regression, investigators in a large multicenter study of children with atopic dermatitis⁽¹⁸⁾ observed that nonspecific influences such as exposure to gas cooker emissions and tobacco smoke increased total IgE levels. They suggested that measurement of this marker alone may not be sufficient in infants. Measurement of specific IgE antibody levels to food and environmental allergens, within the constraints of available blood sample quantity, may be more appropriate in our study group. Transient low-level specific IgE responses to food and then later to inhaled allergens have been observed in young infants but are then downregulated in nonallergic children, while levels in children who develop allergy persist for a longer period.⁽¹⁹⁾

β LG IgG antibody median levels were highest at 4 months corrected age in all infants, reflecting the use of formula feeds—all infants were fed a combination of breast and formula milk before weaning. These levels had decreased by 12 months corrected age. This pattern is similar to that found by Jenmalm and Björkstén⁽²⁰⁾ in AGA infants, in whom levels peaked at 6 months and then decreased to 18 months and even further by 8 years. This group also found similar β LG IgG levels in infants from atopic and nonatopic backgrounds, although they did see a trend for higher IgG levels and significantly higher IgG4 in cord blood of infants from atopic backgrounds. Holt and colleagues⁽²¹⁾ suggested that the doses of food allergens ingested by infants are sufficient to induce specific anergy and thereby “tolerance.”

OVA IgG antibody levels dropped initially at 4 months corrected age, illustrating the degradation of maternal IgG transferred via the placenta, and the lack of egg and egg products in the diet to induce an antibody response. Levels of OVA IgG then increased at 12 months corrected age, reflecting the introduction of egg-containing foods to the diet. IgG antibodies to OVA were seen in all infants, although levels were higher in infants with an atopic family history. This is consistent with the work of Barnes et al.⁽²²⁾ who found IgG antibodies to ovalbumin in infants both with and without atopic eczema (family allergy history not specified) in a cross-sectional study of children between 1 and 16 years of age.

A larger sample size might allow the finding of a higher frequency of allergic disease in SGA infants at 12 months corrected age to be further investigated. It would also permit separate examination of children diagnosed as allergic or nonallergic at 12 months corrected age, from different atopic backgrounds. Jenmalm and Björkstén⁽²⁰⁾ found higher levels of β LG IgG at 6 months of age in full-term infants who developed atopic disease, although levels were similar by 8 years. All the infants were exposed to cow's milk formula as well as breast milk from an early age and were weaned at a similar age. It would be interesting to investigate in a SGA cohort whether late exposure to cow's milk, i.e., exclusive breast-feeding for 3–4 months, or an inappropriate weaning regimen altered their immunological profile.

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