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**High salivary secretory IgA antibody levels are associated with less late-onset wheezing  
in IgE-sensitized infants**

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## **ABSTRACT**

### **Background**

Low levels of secretory IgA (SIgA) and transient IgA deficiency have been associated with an increased risk for allergy, but data is conflicting.

### **Objective**

To assess the relationship between salivary SIgA antibody levels at one year and wheezing at age four in a birth cohort, in particular the possible protective role of salivary SIgA in sensitized children.

### **Methods**

Saliva samples were obtained from all children (n=67) with a positive skin prick test (SPT) at one year and 212 children with a negative SPT. In all, 200 of these children responded to questionnaires at four years and 183 were skin prick tested at that age. The levels of salivary SIgA and salivary IgA antibodies to the most common food allergen egg and inhalant allergen cat were analyzed by ELISA. Serum was analyzed for IgE antibodies to egg and cat.

### **Results**

Development of late-onset wheezing was associated with low SIgA levels in children with positive SPT to at least one allergen both at one and four years of age (p=0.04), as well as in children with circulating IgE antibodies to egg or cat at one year (p= 0.02). None of nine persistently sensitized children with SIgA levels in the upper quartile developed wheezing, as compared to 10/20 children with lower levels (p=0.01). Older siblings, more than three

infections during infancy, at least one smoking parent and male gender were all associated with SIgA in the upper quartile.

**Conclusion** High levels of SIgA antibodies in sensitized infants were associated with significantly less late-onset wheezing, supporting a protective role against development of asthmatic symptoms. Recurrent infections and other factors supporting an increased microbial pressure during infancy were associated with high levels of salivary SIgA.

*Key words:*

Secretory IgA, saliva, sensitization, late-onset wheezing, children

## **ABBREVIATIONS**

IgA	Immunoglobulin A
IL	interleukin
pIgR	polymeric Ig receptor
SC	secretory component
SIgA	secretory IgA
SPT	skin prick test

## INTRODUCTION

Immune defenses against pathogens, but also unresponsiveness (tolerance) are key features of the mucosa, as a vast number of ingested and inhaled food and commensal bacterial antigens must be tolerated (1). While the neonatal mucosa is endowed with all major elements of innate and adaptive immune systems, some functions are relative deficient (2-4). This may prevent both expression of active immune responses and development of tolerance (5).

The primary functional role of IgA antibodies is to provide a first line of defense against a wide variety of pathogens by preventing attachment of bacteria or toxins to epithelial cells, as well as absorption of foreign substances (6). Plasma cells in the mucosal lamina propria secrete dimeric IgA associated with a single J chain (6). This form of IgA binds to the polymeric Ig receptor (pIgR) on the basolateral surfaces of the overlying epithelial cells. The bound complex then undergoes transcytosis, *e g* transportation in a vesicle across the cell to the apical surface, where the extracellular ligand-binding region of pIgR is cleaved and released. SIgA is resistant to cleavage by proteolytic enzymes and therefore well suited for surface protection. Mucosal SIgA antibody responses are non-inflammatory and induced by immunoregulatory and IgE-inhibitory cytokines, such as TGF- $\beta$  and IL-10 (6).

Secretory IgA antibodies prevent adherence and penetration of antigens and high levels could theoretically prevent allergen absorption while low levels of SIgA and transient IgA deficiency have been associated with an increased risk for allergy and bronchial hyperreactivity (7-9). Other studies indicate that high levels of total IgA (10, 11) and IgA antibodies to inhalant allergens (10) are associated with an increased risk of allergic sensitization, while sensitized infants with no clinical symptoms had higher levels of SIgA

than sensitized symptomatic infants (10). Possible explanations for the divergent findings could be the age of the study group, status of sensitization and possible differences between total IgA and SIgA antibodies.

The aim of this study was to assess the relationship between salivary SIgA levels at one year of age and late onset wheezing at four years of life in children from a large birth cohort, in particular a potentially protective role of SIgA in sensitized children. We also assessed the association between SIgA antibody levels and environmental factors encountered early in life.

## METHODS

### *Subjects*

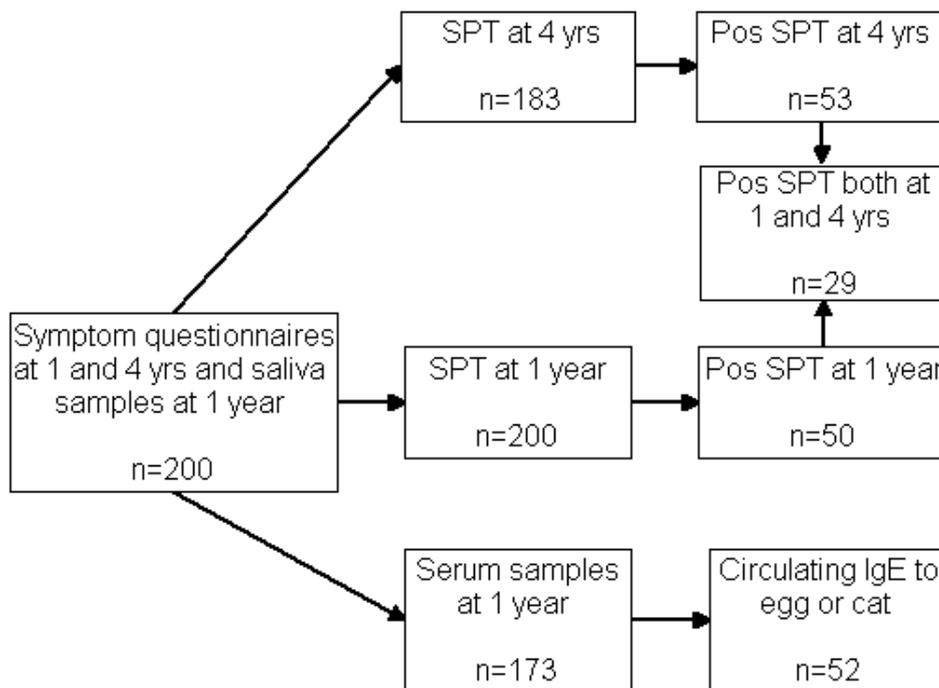
The children participated in a birth cohort study comprising 1231 children born between February 1996 and January 1997 in Östersund, a town in the mountain region of central Sweden (12). Skin prick tests (SPT) were performed at one year of age in 1040 children. All children with at least one positive SPT (n=71) and a random sample of three SPT negative children (n=213) with birthdates close to that of each index child, in all 284 children, were invited to an interview and a clinical examination by a pediatrician (AS). Saliva samples were obtained from 279 children, of whom 67 had at least one positive SPT and 212 were SPT negative. A questionnaire reported history of allergy among members of the immediate family was given in 43% of the infants, 64% reported three or more infections (not specified) during infancy and 71% had been exclusively breastfed up to four months. At four years, 200/279 children replied to questions concerning symptoms and environmental exposures and 183 of them were also skin prick tested. Serum samples obtained at one year were available from 173 of the 200 children (Fig. 1).

The rates of breastfeeding for more than four months, number of older siblings, passive smoking and family history of allergic disease were similar in the 200 participants and the 79 dropouts.

### *Skin prick tests and determination of IgE*

Skin prick tests were performed at one and four years of age with standardized extracts of five allergens, *i.e.* egg, milk, birch, timothy and cat (Solu-Prick SQ, ALK-Abelló, Hørsholm, Denmark). Histamine hydrochloride (10mg/mL) was used as a positive control and the test

was considered positive if the mean wheal diameter was at least three mm after 15 minutes. The serum samples were stored at  $-20^{\circ}\text{C}$  and analyzed for specific IgE antibodies to egg and cat (Pharmacia CAP System Specific IgE FEIA, Pharmacia Diagnostics, Uppsala, Sweden). The cut-off level was 0.35 kU/L.



**Fig 1 Flow chart:** Skin prick test results and circulating IgE antibodies in 200 children who delivered saliva samples at one year and answered to questionnaires at both 1 and 4 years.

#### *Saliva and determination of IgA*

Saliva samples were collected in a standardized way from a region in the mouth close to the lateral mucous membrane, using a vacuum-pump connected to a thin plastic tube. The children were non-fasting and there was no prior stimulation of the salivary flow rate. A visual inspection revealed that there was no inflammation or bleeding from the oral cavity. The samples were immediately frozen at  $-20^{\circ}\text{C}$  until analysed. Long time storage has earlier been reported not to have any effect on the antibody affinity (13), nor does long time storage and repeated cycles of freezing and thawing alter the molecular weight of IgA (14). The levels of salivary SIgA, as well as salivary IgA antibodies to egg and cat (one food and one airborne

antibody) were determined by ELISA. This method has been described in detail previously where the specificity of the assay has been confirmed by inhibition tests and the lack of demonstrable IgA in five samples from IgA-deficient individuals (10). For detection of SIgA, the plates were coated with an anti-human secretory component antibody (Dakopatts AB), detecting only SIgA.

### *Definitions*

*Persistent wheezing* was defined as wheezing both at one and four years of age.

*Late-onset wheezing* was defined as wheezing between three and four years of age, but not at one year.

### *Statistics*

All the data were coded and entered into the database module of the SPSS statistics software (SPSS Inc, Chicago, IL, USA). As the concentrations of salivary IgA antibodies were not normally distributed, comparisons between unpaired groups were analyzed with Mann-Whitney U-test. Salivary antibodies were also analyzed as a dichotomized variable using the 75<sup>th</sup> percentile as a cut-off level. Chi-square test was used to assess association between categorical variables. Fischer's exact test was used when the expected frequency for any cell was less than five. A p-value < 0.05 was considered significant. Multivariate logistic regression analyses were used to obtain estimates of determinants for high levels of SIgA with adjustments for significant co-variables.

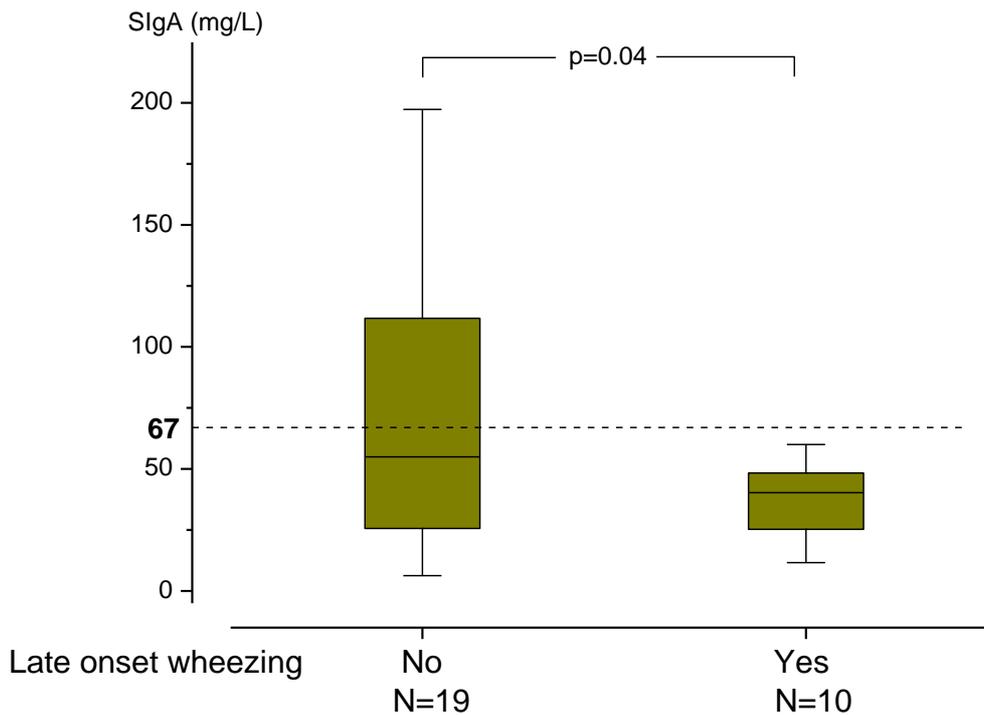
### *Ethical considerations*

The study was approved by the Regional Ethical Committee for Human Research at the University Hospital, Umeå. Parental consent was obtained separately for each part of the study.

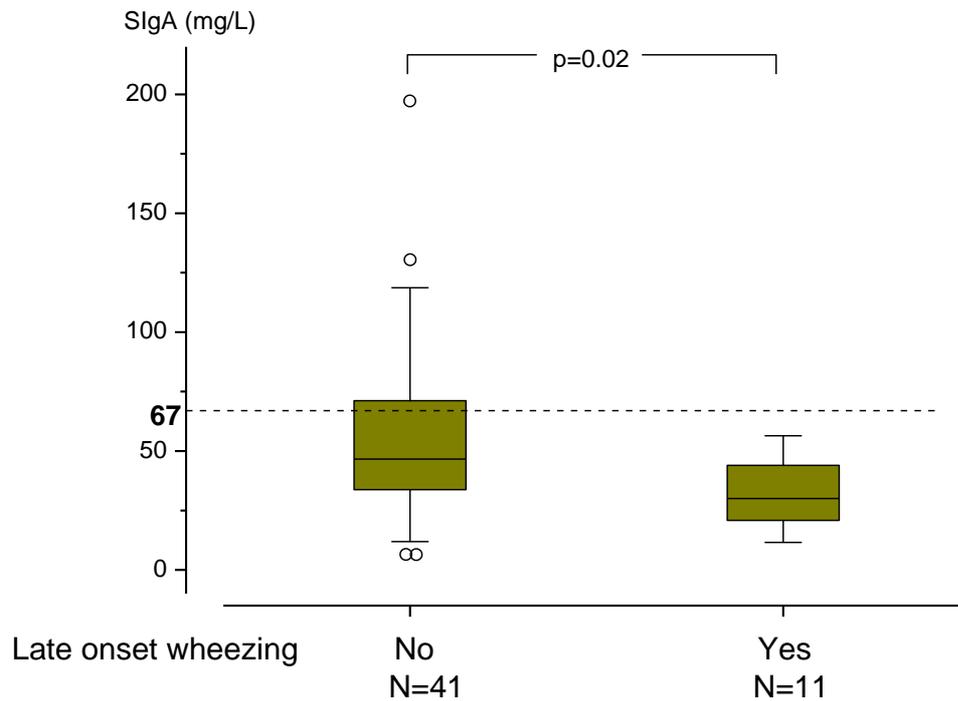
## RESULTS

The median levels of SIgA in the saliva were 45 mg/L (ranges 5-197, upper quartile above 67). Twenty-two children reported wheezing both at one and four years (persistent wheezing) and 26 children only at four years (late-onset wheezing). High levels of SIgA (in the upper quartile) were associated with less late-onset wheezing in sensitized children. In children with a positive SPT at four years, none of 14 children with high levels of SIgA had late-onset wheezing, as compared to 12/39 children with lower levels (Chi-square,  $p=0.02$ ). In children with positive SPT both at one and four years, SIgA levels were lower in those who developed late-onset wheezing than in those who did not (Mann-Whitney U-test,  $p=0.04$ , Fig 2A). Among children with persistent sensitization, none of nine children with high levels of SIgA developed wheezing, as compared to 10/20 children with lower SIgA levels (Chi-square test,  $p=0.01$ ). There was no association between SIgA levels and current wheezing at one year (data not shown).

We also assessed sensitization based on circulating IgE antibodies to egg and cat, the two most common allergens in this age group. Among children with circulating IgE antibodies to egg and/or cat at one year, those who developed late-onset wheezing had lower levels of SIgA than those who did not (Mann-Whitney U-test,  $p=0.02$ , Fig 2B). None of 13 infants with SIgA levels in the upper quartile in addition to circulating IgE antibodies to egg or cat developed late-onset wheezing at four years, as compared to 11/39 similarly sensitized infants with lower levels of SIgA (Chi square test,  $p=0.05$ ). There was no association between SIgA levels and persistent wheezing (data not shown).



**A**



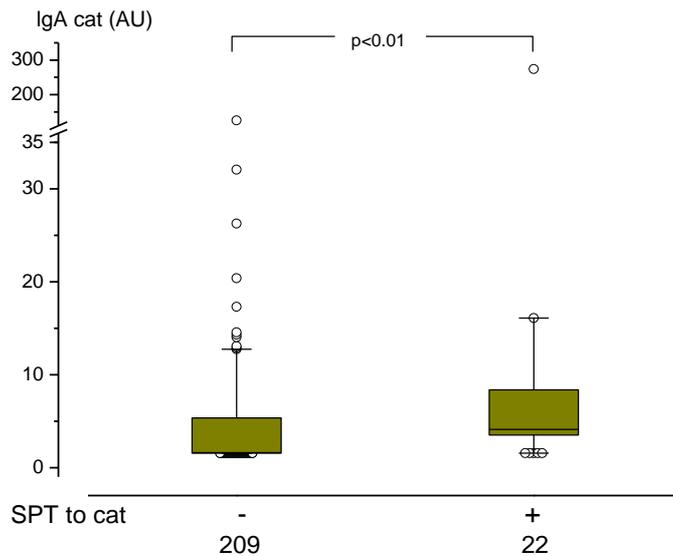
**B**

**Fig. 2 SIgA antibody levels in sensitized children**

SIgA antibody levels in sensitized children were lower in those who developed late onset wheezing than in those who did not. Children who were SPT positive both at one and four years of age are included in (A) and children with circulating IgE antibodies to egg and/or cat at one year of age in (B).

In the boxes 25<sup>th</sup>, 50<sup>th</sup>, and 75<sup>th</sup> quartiles are indicated, as well as outliers. The stippled line marks the upper quartile (above 67 mg/L) for all 279 saliva samples.

Immunoglobulin A antibodies to cat were detected in 120/231 (52%) and to egg in 193/257 (75%) of the saliva samples. There was no relationship between wheezing symptoms at one or four years and IgA antibodies to egg or cat. High salivary IgA antibody levels to cat were detected in infants with positive SPT to cat at one year (Fig 3), but there was no association between salivary IgA antibodies to egg and any sensitization.



**Fig. 3 IgA antibodies to cat**

The levels of IgA antibodies to cat in saliva were higher in children with positive, as compared to negative SPT to cat. The 25<sup>th</sup>, 50<sup>th</sup>, and 75<sup>th</sup> quartiles are indicated, as well as outliers.

Older siblings, more than three infections during infancy, at least one smoking parent and male gender were all associated with SIgA levels in the upper quartile (Table). The findings were similar in sensitized and non-sensitized children.

## DISCUSSION

Children with SIgA levels in the upper quartile at one year of age were less likely to develop late-onset wheezing at four year of age, as compared to sensitized infants with lower levels of SIgA. Our findings are in line with another study, where SPT-positive infants who developed allergic symptoms during the first two years of life had lower levels of SIgA in saliva at one year of age (10). We have extended this observation and demonstrate a protective role of salivary IgA against development of wheezing in sensitized children.

The protective effect of higher SIgA among sensitized children was true both for persistent sensitization defined as a positive SPT to at least one of five common allergens (milk, egg, cat, timothy and birch), as well as for sensitization at one year defined as detectable circulating IgE antibodies to the most common food allergen and airborne allergen during childhood (egg and cat). As there is negligible house dust mite sensitization in northern Sweden (15), this allergen was not included in the study.

We have previously demonstrated a five-fold increased risk of late-onset wheezing in this cohort among children who were sensitized at one year of age (12). We now show that high levels of SIgA at one year may protect this group of sensitized high-risk children from developing late-onset wheezing. The presence of secretory components enhance stability and effector functions of IgA (16). High levels of SIgA in the mucosa could possibly interfere with the interaction between allergen and IgE antibodies in sensitized individuals, thereby preventing triggering of allergic inflammation and clinical symptoms. This notion is supported by animal models showing that mucosal IgA responses inhibit airway reactivity in sensitized mice (17-19). Even though our study was based on all children in a birth cohort of

1231 children, the number of sensitized children was small. Therefore, we have to be cautious in the interpretation of our findings. It is striking, however, that none of the 14 children with levels of SIgA in the upper quartile developed symptoms of late-onset wheezing at 4 years of age even though they were sensitized during infancy and thus at high risk.

The levels of IgA antibodies to cat were higher in children with a positive as compared to a negative SPT to cat. A previous Swedish study also demonstrated higher levels of IgA antibodies to allergens in allergic children (10). Similarly, high levels of IgA antibodies to ragweed were more likely in nasal secretions from patients with hay fever (20). Furthermore, the levels of IgA antibodies to mite in saliva were higher in asthmatic adults with positive SPT to house dust mite, than in saliva from non-atopic healthy controls (21). In contrast, we did not find any association between sensitization to the food allergen egg and salivary levels of IgA antibodies to egg. This is possibly due to different doses and routes of exposure of inhalant and food allergens, since IgA antibodies to inhalant allergens such as mite, ragweed and cat are predominantly locally produced (22).

Older siblings, passive smoking and infections during infancy were all independent determinants of high levels of SIgA. An inverse relationship between sibship size and the risk of allergic disease has been a consistent finding in many studies. Secretory IgA did not affect the rate of sensitization but seemed to interfere with development of wheezing symptoms in sensitized children, hypothetically through increased activity of Treg cells and/or inhibition of the interaction between allergens and IgE antibodies in sensitized individuals. Having older siblings and a history of more than three infections are environmental factors associated with high microbial load stimulating the immune system. We have previously in the same birth cohort suggested that dog keeping during the first year of life, possibly associated with an

increased microbial pressure (23), might provide some protection from late-onset wheezing (12). Moreover, we recently demonstrated that an increased bifidobacterial diversity during the first two months of life was associated with enhanced salivary SIgA levels in Swedish infants (24). Maternal smoking and an increased incidence of respiratory and gastrointestinal infections have previously been associated with higher levels of salivary IgA in one-year old children (25).

In conclusion, high levels of SIgA antibodies in sensitized infants were associated with significantly less late-onset wheezing at four years of age, supporting a protective role of such antibodies. The high levels of SIgA antibodies in infants with older siblings and recurrent infections are of particular interest in the light of numerous reports relating environmental exposures, microbiological load and childhood allergic symptoms.

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