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Early life EBV-infection protects against persistent IgE-sensitization

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ABSTRACT

Background: Infection with Epstein-Barr virus (EBV) has previously been implicated in influencing allergic disorders but its precise role remains contradictory. The timing of primary infection may contribute to the discrepancies. This study aimed at investigating whether the time-point of primary EBV infection during childhood could be of importance in modulating the risk of developing IgE-sensitization.

Methods: A total of 219 Swedish infants were followed prospectively to five-years of age with clinical examinations, skin prick testing, specific IgE analyses and determination of serostatus against EBV.

Results: Following analysis of the children's EBV serostatus, we found that five-year olds who were infected with EBV before the age of two were at a significantly lower risk of being persistently IgE-sensitized, i.e. sensitized at both two- and five-years of age (adjusted odds ratio 0.34; 95% CI 0.12-0.94). In contrast, contraction of EBV after two-years of age was highly associated with late-onset IgE-sensitization (adjusted odds ratio 4.64; 95% CI 1.57-13.69). Persistently sensitized five-year olds had higher specific-IgE levels than children with late-onset IgE-sensitization ($p<0.01$).

Conclusions: Our data supports the value of early-life microbial exposure for protection against the development of IgE-sensitization, and underscores the proximate postnatal years as an important period during which EBV could contribute to an allergeo-protective immune profile.

Clinical Implications

Appropriate microbial immune stimulation at a very young age may overcome maturational deficiencies in the immune system and allergy development. EBV could represent one such stimulatory agent.

Capsule summary

EBV infection before the age of two significantly relates to protection against persistent IgE-sensitization. In contrast, contraction of EBV after the age of two is a risk factor for IgE-sensitization later in childhood.

Key words

Epstein-Barr virus; seronegative; seropositive; age-dependent dual roles of infection; polyclonal stimulatory effect; persistent IgE-sensitization

Abbreviations

CMV: Cytomegalovirus

EBV: Epstein-Barr virus

NK cells: Natural killer cells

OR: Odds ratio

SPT: Skin prick test

INTRODUCTION

The underlying reasons for the increased prevalence of allergic disorders among the world's affluent populations are largely unknown. A decrease in particular childhood infections or a lower exposure to a diverse range of infectious agents during infancy has been suggested as contributing factors¹. An altered overall spectrum of infections may set the stage for an imbalanced T-helper type 1 (Th1)/Th2-immune profile, which could contribute to the development of allergies^{2,3}.

Epstein-Barr virus (EBV) and cytomegalovirus (CMV) are herpesviruses that are widely spread in human populations. A general route of CMV infection in early childhood is through the mother's genital secretions at birth and through breast-milk⁴, while EBV transmission commonly occurs via saliva^{5,6} from family members. After primary infection, EBV and CMV establish latency, persist and are chronically or intermittently shed for the lifetime of the host^{5,6}. Variations in EBV and CMV seroprevalence are associated with socioeconomic status; approximately the whole population in developing countries becomes seropositive before adolescence while children from industrialized countries appear to have a delayed primary infection^{4,7}.

Infections with EBV and CMV have previously been implicated in influencing allergic disorders but their precise role remains controversial. Some studies have suggested allergy protective^{8,9} or -promoting¹⁰⁻¹² effects, while others could not demonstrate a clear role for EBV or CMV in the pathogenesis of allergic disorders^{13,14}. A previous study from our group showed an inverse relation between EBV infection and IgE-sensitization in a cohort of Swedish two-year old infants⁹. We also observed that seropositivity with CMV in the absence of EBV tended to associate with a higher risk of IgE-sensitization. This suggests that infection with EBV during the two first years of life may protect from allergy. We have also recently

demonstrated that both EBV and CMV might influence early-life development towards diverse immune profiles¹⁵.

The current study aimed to further investigate whether the time-point of primary EBV infection during childhood could be of importance in protection against development of sensitization. This question was addressed by re-examination of the study cohort⁹ at five-years of age. Following analysis of the children's EBV and CMV serostatus, we demonstrate that early-life EBV infection significantly relates to protection against persistent IgE-sensitization. Further, in sharp contrast to the group that was infected before the age of two, those subjects that contracted EBV after the age of two were at a markedly higher risk of becoming IgE-sensitized later in childhood.

METHODS

Study population and determination of serostatus against EBV and CMV

This study includes 219 children (table 1) born between 1997 and 2000 in Stockholm that are part of a prospective study cohort (n=281). One third of the children had two allergic parents, another third had an allergic mother and the remaining third had no parental history of allergy. Positive or negative history of parental allergy was confirmed with skin prick tests (SPTs) before delivery of the child. A detailed description of the cohort has previously been published⁹. All infants were healthy and born full term (>36 weeks of gestation; mean 40, range 36-43) at hospitals in Stockholm. The birth weights ranged between 1.7-4.8 kg with a mean of 3.6. The presence of plasma IgG against EBV capsid antigen (EBV VCA) was determined according to previously published immunofluorescence assays¹⁶ (where a specific fluorescence in dilution 1/20 was regarded as a sign of seropositivity) and by a peptide ELISA for EBV nuclear antigen 1¹⁷. IgG seropositivity to CMV was determined by ELISA using purified nuclear antigens from CMV cultivated in human fetal lung fibroblasts¹⁸, where

samples with an absorbance above 0.2 at a dilution of 1/100 were considered positive. Since measurement of circulating IgE was given higher priority and the volumes of plasma samples were sometimes too small, the serological data at the age of five are as follows: both EBV and CMV serostatus was determined in 192 out of 219 five-year olds and data for CMV serostatus was determined in 195 (three additional subjects) out of 219 five-year olds. The study was approved by the Human Ethics Committee at Huddinge University Hospital, Stockholm, and the parents provided informed consent.

Skin Prick Testing and determination of circulating allergen-specific IgE

The children underwent SPT against food and inhalant allergens, which were performed according to the manufacturer's recommendation (ALK, Copenhagen, Denmark). The SPT, at two and five years of age, included food allergens: egg white (Soluprick weight to volume ratio 1/100); cod (Soluprick 1/20); peanut, (Soluprick 1/20); cow's milk (3% fat, standard milk) and soybean protein (Soja Semp® Semper AB, Stockholm, Sweden). SPT was also performed for inhalant allergens: cat; dog; *Dermatophagoides farinae*; birch and timothy (Soluprick 10 HEP). In addition to aforementioned allergens, subjects were also tested for rabbit at the age of five (Soluprick 10 HEP). The SPT was considered positive if the wheal diameter was ≥ 3 mm after 15 minutes. Histamine chloride (10 mg/ml) and the allergen diluent served as positive and negative control respectively. The children were serologically tested for IgE against the same allergens as those used for SPT, all performed with ImmunoCAP™ (Phadia AB, Uppsala, Sweden). The test was considered positive if allergen-specific IgE levels were ≥ 0.35 kU_A/L.

Clinical evaluation and classification of IgE-sensitization

Subjects were followed from birth until 60 months of age and were clinically examined by one paediatrician (C.N.) at ages six, 12, 18, 24 and 60 months. In accordance with Johansson et al.¹⁹ the child was classified as IgE-sensitized if at least one or more SPT was positive (≥ 3 mm) and/or if specific IgE against at least one or more of the selected allergens was $\geq 0.35 \text{ kU}_A/\text{l}$. A total of 225 children (80%) were eligible for evaluation of IgE-sensitization at both two- and five-years of age. Among these, six children were excluded since the results of the EBV analyses were indeterminate despite repeated examination or due to insufficiency of plasma volumes for performance of additional analyses. The remaining 219 subjects were classified according to presence or absence of IgE-sensitization as follows: non-sensitized (neither IgE-sensitized at two- nor at five-years of age), $n=129$ (59%); transiently sensitized (IgE-sensitized at two- but not at five-years of age), $n=7$ (3%); persistently sensitized (IgE-sensitized at both two- and five-years of age), $n=48$ (22%) and late sensitized (IgE-sensitized at five- but not at two-years of age), $n=35$ (16%, table 1).

Statistical methods

Odds ratio (OR) estimates and corresponding 95% confidence intervals (95% CI) for development of IgE-sensitization were obtained by logistic regression. Data were adjusted for the following background variables: sex, parental allergy (none, maternal or both parents), maternal age, duration of breast-feeding, furred pets, month of birth, older siblings and parental smoking by multiple logistic regression analysis. Mann-Whitney U-test was used to assess differences in IgE-levels between sample groups. Fisher's exact test was applied to evaluate differences between the transiently sensitized and non-sensitized group and to test differences in susceptibility to EBV or CMV infection between sensitized and non-sensitized subjects. Statistical analyses were performed at the Division of Mathematical Statistics, Stockholm University. P-values below 0.05 were considered statistically significant.

RESULTS

IgE-sensitization among five-year olds

Among the 219 five-year olds, 83 (38%) were classified as IgE-sensitized. In the group of persistently IgE-sensitized subjects (n=48), 37 (77%) were SPT positive while 46 children (96%) had a positive test for circulating IgE against one or more of the selected allergens. Corresponding data for the late-sensitized group (n=35), was 21 (60%) for SPT and 34 children (97%) for circulating specific IgE. The group of persistently sensitized children had higher levels of allergen-specific IgE compared with late-sensitized subjects ($p<0.01$, Fig 1). No significant differences were seen between non-sensitized, persistently sensitized and late sensitized children with regard to maternal age, birth weight, gestation week, maternal or paternal smoking (data not shown). We observed, however, that IgE-sensitized children were significantly more likely to have two allergic parents and that the group of persistently sensitized children was more likely to be born during the winter than late sensitized and non-sensitized children. Further, delivery by caesarean section was more common among late sensitized children (Table 1). The group of transiently IgE-sensitized children (n=7), did not differ from the non-sensitized group regarding any of the investigated demographic parameters (data not shown).

EBV and CMV seroprevalence among the two- and five-year olds

Data for the whole cohort showed that among the 219 two-year olds, 109 (50%) children were seronegative for both EBV and CMV, 25 (11%) were seropositive for EBV alone, 66 (30%) were only CMV seropositive. The remaining 19 (9%) were seropositive for both herpesviruses. Corresponding results for five-year olds (n=192) demonstrated that 30 (16%) children were seronegative for both EBV and CMV, 20 (10%) were seropositive for EBV

alone, 89 (46%) were only CMV seropositive and the remaining 53 (28%) were seropositive for both herpesviruses (data not shown in table).

Relation between IgE-sensitization and EBV and CMV infection during early childhood

We examined the frequency of EBV and CMV seropositivity in relation to IgE-sensitization during infancy and childhood (Table 2). The transiently sensitized group was excluded from these analyses since it contained too few subjects ($n=7$) for a reliable statistical outcome. As the number of subjects with determined serostatus differed between the two and five year olds, table 2 was divided in section A; where relation between serostatus at age two and persistent IgE-sensitization was evaluated, and section B; where serostatus at two or between two and five years of age was related to late IgE-sensitization. We found that the five-year old children who were EBV seropositive at two-years of age were at a decreased risk of being persistently IgE-sensitized (adjusted odds ratio, OR_{adj} 0.34; 95% CI 0.12-0.94). This association was not observed for CMV infection, which rather tended to be associated with an increased risk (OR_{adj} 1.66; 95% CI 0.84-3.31). In sharp contrast to EBV-infection before the age of two, EBV seropositivity acquired between the ages of two and five was related to a significantly higher risk of having developed IgE-sensitization at five-years of age (late IgE-sensitized, OR_{adj} 4.64; 95% CI 1.57-13.69). No such association was observed for CMV infection. The frequency of early EBV infection, i.e. seropositivity at two-years of age, did not differ between late sensitized and non-sensitized five-year old children (OR_{adj} 1.36; 95% CI 0.47-3.92), which suggests that early infection with EBV does not protect against late IgE-sensitization. No protective effect was observed when persistently and late-sensitized subjects were regrouped into one group and compared with the non-sensitized children (data not shown).

To evaluate if the exclusion of these children influenced the above findings, we regrouped the transiently sensitized and the persistently sensitized subjects into one group, given that the children in both groups were IgE-sensitized at two-years of age. However, we observed no noteworthy changes in the results when these children were grouped together (OR_{adj} 0.37; 95% CI 0.14-0.95 for transient plus persistent, not displayed in table).

Relation between IgE-sensitization and susceptibility to EBV and CMV

We also addressed whether susceptibility to EBV or CMV is altered in IgE-sensitized subjects. To this end, we excluded two-year olds who were seropositive for both EBV and CMV, divided the remaining children into IgE-sensitized or non-sensitized and analyzed the frequency of EBV and/or CMV seropositivity at five-years of age. There were no differences in the proportion of subjects who seroconverted for EBV and/or CMV between the IgE-sensitized and non-sensitized group (Table 3), suggesting that acquisition of EBV and CMV is not influenced by the infant's allergic status.

DISCUSSION

This study demonstrates that acquisition of EBV infection during early-life associates with a reduced risk of persistent IgE-sensitization. This effect seems to be limited to a relatively short time period during infancy, as infection with EBV after two-years of age relates to a significantly higher risk of IgE-sensitization. However, EBV infection during infancy does not confer protection against IgE-sensitization later in childhood. Interestingly, we observed markedly higher allergen-specific IgE levels among persistently IgE-sensitized children than among late sensitized children. This could set the stage for the development of atopic phenotypes as it has been shown that persistent atopic sensitization with an early-onset

confers a higher risk for developing allergic asthma/wheezing later in life than late-onset sensitization^{20,21}.

The concept of an “early window of opportunity” for protection against allergy underscores the importance of exposure to pathogenic and non-pathogenic microorganisms during the earliest periods of life for proper shaping of the immune system away from the allergic phenotype²². Our data indicates that early-life EBV infection may contribute to this immunomodulation and suggests that examination of defined age groups might overcome contradictions between previous reports on EBV’s relation to allergy. *Calvani et al* observed that EBV infection during early childhood (especially <29 months) was related to protection against atopy, as opposed to primary EBV-infection in older children⁸. In the study of *Sidorchuck et al*, where no association could be demonstrated, 4-year old children were investigated¹³, which in relation to our findings would be children both exposed to the protective effect before two years and the aggravating effect thereafter. In studies where EBV infection has been pointed out as a risk factor in atopy the investigated subjects were either between five and 18-years old¹⁰ or adults¹¹, further supporting the age-dependent protective role of EBV infection.

A possible mechanism behind the protective effect of early-life EBV infection might be the polyclonal stimulatory effect of EBV on the immune system. While primary, symptomatic, EBV-infection is accompanied by a vigorous T-cell response and production of a multitude of antibodies⁵; the strength of such infection in asymptomatic children is unknown. However, elevated levels of both Th1 and Th2 cytokines have previously been demonstrated in two-year old EBV-seropositive infants¹⁵, which could be a marker of a stimulatory effect of EBV. Also, during ongoing symptomatic EBV-infection, heterophile antibodies (IgM antibodies against heterologous erythrocyte-antigens for example from horse and ox) are readily detected, while these are usually absent in asymptomatic infants with

primary EBV infection²³. Absence of B-cells primed against the heterophile antigens due to lack of sufficient antigen exposure at an early age may be an explanation. In analogy, in the absence of an allergen-specific memory pool during early postnatal years, the mitogenic effect of EBV would not precipitate an allergy-promoting immune profile. If allergen-specific, IgE primed cells already exist however, as in children sensitized early in life, a subsequent EBV infection could promote expansion of these cells. This may explain our observation of an increased risk for IgE-sensitization associated with EBV infection after the age of two and indicates that EBV has an age-dependent role in the modification of the risk to develop IgE-sensitization.

It has been suggested that infants predisposed to allergies require a strong and adequate immune stimulation at a very young age to overcome maturational deficiencies in their immune system²⁴. EBV, presumably in addition to other suggested microbial candidates such as intestinal bacteria²⁵, might represent one such stimulatory agent. Indeed, the majority of children below 3-years of age are infected with EBV in developing countries⁵ where the occurrence of allergic disorders is uncommon²⁶. However, the asthma prevalence in those countries seems to rise now and increased industrialization has been proposed as a causing factor²⁷. Other viral agents have been discussed in relation to allergy, both as being protective and as risk factors²⁸. For instance, Hepatitis A has been shown to inversely correlate to the prevalence of atopic diseases and Measels has been suggested to protect against IgE sensitization but the results are contradictory²⁹. In the case of respiratory syncytial virus (RSV) infection, it appears that infants who require hospital care have a higher prevalence of asthma and allergy compared to children without severe symptoms³⁰. Our data here does not support a risk modulation by CMV, which is in agreement with previous observations^{9, 13}.

Collectively these observations suggest that not only viral infections *per se*, rather the type of infecting agent might be of importance in the modulation of the risk to become IgE- sensitized.

The quality of innate and acquired responses in the two-year olds from this cohort has been previously reported. Interestingly, we found that EBV seropositivity associates with a lower monocyte-dependent IFN- γ release by NK cells³¹ whereas T-cell cytokine responses tend to be enhanced¹⁵. Therefore, an alternative, or additive, mechanism behind the protective effect of early EBV infection could be that attenuated innate responses facilitate a general boost of adaptive immunity.

We should acknowledge some study limitations. In agreement with other reports^{20,21}, the subjects in our study were classified into four sensitization groups, where the transiently sensitized included very few subjects. The exclusion of the transiently IgE- sensitized group from the analyses of EBV's relation to protection against persistent IgE- sensitization could have biased our results, but since regrouping of the transient group with the persistent did not alter the outcome of our findings this is unlikely. Further, the seroprevalence of EBV (38%) among five-year olds in this cohort was lower than described for a previous cohort of 2561 Swedish children from the whole Stockholm region, where 53% were EBV seropositive at the age of 4¹³. Our smaller cohort size and that all participants were recruited from a limited part of Stockholm might be possible explanations for the discrepancy. Since two assays for EBV seropositivity were combined in our study, we believe that our evaluation of seropositivity is highly accurate. It should be stressed that the purpose of this study was not to examine EBV seroprevalence, but the relation between infection with EBV and CMV and IgE-sensitization.

Conclusion

Findings from this study support a protective role for EBV-infection before two-years of age in relation to persistent IgE-sensitization in childhood. Whether this group displays a differential atopic phenotype later in life compared to the late-onset group in our cohort remains to be determined; a 10-year follow up is ongoing.

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Table 1. Five-year old subject demographics.

	Total [¶]	Non-sensitized ^{†‡}	Persistently IgE-sensitized ^{#†}	Late IgE-sensitized ^{§‡}	OR; 95% CI [†]	OR _{adj} ; 95% CI [†]	OR; 95% CI [‡]	OR _{adj} ; 95% CI [‡]
n (%)	219	129 (58.9)	48 (21.9)	35 (16.0)				
Sex								
boy; n (%)	114 (52.1)	68 (52.7)	27 (56.3)	15 (42.9)	1	1	1	1
girl; n (%)	105 (47.9)	61 (47.3)	21 (43.8)	20 (57.1)	0.87; 0.45-1.69	0.86; 0.39-1.88	1.49; 0.70-3.16	1.16; 0.50-2.69
Allergic heredity								
Non heredity; n (%)	62 (28.3)	47 (36.4)	8 (16.7)	6 (17.1)	1	1	1	1
Double heredity; n (%)	90 (41.1)	40 (31.0)	28 (58.3)	19 (54.3)	4.11; 1.69-10.03	6.16; 2.17-17.47	3.72; 1.36-10.22	7.95; 2.21-28.54
Maternal heredity; n (%)	67 (30.6)	42 (32.6)	12 (25.0)	10 (28.6)	1.68; 0.63-4.50	2.06; 0.68-6.20	1.86; 0.62-5.57	3.22; 0.89-11.59
Obstetric data								
Delivery mode								
vaginal; n (%)	183 (83.6)	109 (84.5)	42 (87.5)	26 (74.3)	1	1	1	1
caesarean; n (%)	36 (16.4)	20 (15.5)	6 (12.5)	9 (25.7)	0.78; 0.29-2.07	1.14; 0.36-3.61	1.89; 0.77-4.62	2.99; 1.05-8.52
Birth period								
April-September; n (%)	136 (62.1)	88 (68.2)	22 (45.8)	22 (62.9)	1	1	1	1
October-March; n (%)	83 (37.9)	41 (31.8)	26 (54.2)	13 (37.1)	2.54; 1.29-5.00	3.39; 1.53-7.54	1.27; 0.58-2.77	2.00; 0.81-4.97
Exposure								
Exclusive breast-feeding								
0 months; n (%)	10 (4.6)	6 (4.7)	2 (4.2)	2 (5.7)	1	1	1	1
0.5-3.9 months; n (%)	30 (13.7)	16 (12.4)	10 (20.8)	4 (11.4)	1.88; 0.31-11.17	3.38; 0.43-26.80	0.75; 0.11-5.22	0.70; 0.09-5.43
4.0-5.0 months; n (%)	147 (67.1)	88 (68.2)	28 (58.3)	24 (68.6)	0.95; 0.18-5.00	0.68; 0.10-4.74	0.82; 0.16-4.31	0.51; 0.08-3.09
5.1-10 months; n (%)	32 (14.6)	19 (14.7)	8 (16.7)	5 (14.3)	0.21; 0.31-7.65	1.02; 0.13-8.19	0.79; 0.12-5.17	0.73; 0.10-5.59
Attending day care								
no; n (%)	27 (12.3)	12 (9.3)	9 (18.8)	6 (17.1)	1	1	1	1
yes; n (%)	192 (87.7)	117 (90.7)	39 (81.3)	29 (82.9)	0.44; 0.17-1.13	0.39; 0.13-1.16	0.50; 0.17-1.43	0.33; 0.10-1.10
Day-care start; months*	17.5 (12-26)	18.0 (12-26)	17.0 (12-24)	16.0 (12-25)	0.99; 0.87-1.12	0.95; 0.82-1.10	0.89; 0.77-1.03	0.84; 0.69-1.02
Furred pets; n (%)	40 (18.3)	22 (17.1)	10 (20.8)	8 (22.9)	1.06; 0.45-2.50	1.41; 0.50-3.97	1.37; 0.55-3.39	2.24; 0.76-6.64
Nr of older siblings*					0.62; 0.38-1.02	0.66; 0.38-1.14	0.93; 0.61-1.42	1.07; 0.66-1.74

[¶]Includes the transiently-sensitized group (n=7), which is not displayed separately in the table. [#]IgE-sensitized at 2- and 5-years of age.

[§]IgE-sensitized at 5- but not 2-years of age. [†]Comparison between non-sensitized and persistently IgE-sensitized. [‡]Comparison between non-sensitized and late IgE-sensitized. *Median (range). * Analysis based on continuous variable due to presence of zero-value. n, number; OR, odds ratio; OR_{adj}, adjusted odds ratio for: sex, parental allergy (none, maternal or both parents), maternal age, duration of breast-feeding, furred pets, month of birth, older siblings and parental smoking.

Table 2. Frequency of EBV and CMV seropositivity among IgE sensitized and non-sensitized children.

A	Total	Non-sensitized at 5-years	Persistent IgE-sensitized at 5-years	OR; 95% CI	OR_{adj}; 95% CI
SEROSTATUS At 2-years of age					
n	212	164	48		
EBV+, n (%)	43 (20.3)	38 (23.2)	5 (10.4)	0.39; 0.14-1.04	0.34; 0.12-0.94
CMV+, n (%)	83 (39.2)	61 (37.2)	22 (45.8)	1.43; 0.75-2.74	1.66; 0.84-3.31
B	Total	Non-sensitized at 5-years	Late IgE-sensitized at 5-years	OR; 95% CI	OR_{adj}; 95% CI
SEROSTATUS At 5-years of age*					
EBV serostatus determined, n	146	115	31		
EBV+ at the age of 2, n (%)	32 (21.9)	25 (21.7)	7 (22.3)	1.46; 0.53-4.03	1.36; 0.47-3.92
EBV+ at the age of 5, n (%)	27 (18.5)	17 (14.8)	10 (32.3)	3.07; 1.17-8.08	4.64; 1.57-13.69
CMV serostatus determined, n	147	115	32		
CMV+ at the age of 2, n (%)	53 (36.1)	42 (36.5)	11 (34.4)	0.71; 0.53-4.03	1.36; 0.26-1.89
CMV+ at the age of 5, n (%)	57 (38.8)	46 (40.0)	11 (34.4)	0.65; 0.24-1.72	0.49; 0.17-1.45

The results for the transiently IgE-sensitized children (n=7) are not included in the serostatus at 2 and 5 years of age. *The serostatus at 5 years of age does not include the 48 persistent IgE-sensitized subjects and 18 (for EBV) or 17 (for CMV) missing plasma samples from of the 5-year old material. OR, odds ratio; OR_{adj}, odds ratio adjusted for parental allergy.

Table 3. Relation between IgE-sensitization and susceptibility to EBV and CMV.

Anti viral serostatus at 2-years of age		Anti viral serostatus at 5-years of age		Non IgE-sensitized at 2-years of age (Non- & Late IgE-sensitized)	IgE-Sensitized at 2-years of age (Persistent IgE-sensitized)
EBV	CMV	EBV	CMV	Proportion	Proportion
Negative	Negative	Negative	Negative	0.17	0.15
Negative	Negative	Negative	Positive	0.25	0.20
Negative	Negative	Positive	Negative	0.05	0.05
Negative	Negative	Positive	Positive	0.10	0.03
Negative	Positive	Negative	Positive	0.24	0.35
Negative	Positive	Positive	Positive	0.05	0.13
Positive	Negative	Positive	Negative	0.06	0.05
Positive	Negative	Positive	Positive	0.08	0.05
				n= 132	n= 40
					p=0.53

FIGURE LEGENDS

Figure 1. Allergen-specific IgE levels in five-year old persistently-IgE sensitized (n=46) or late-IgE sensitized (n=35) subjects. Boxes cover values between 25th and 75th percentile, the central square within the boxes represents the median and whiskers span to non-outlier values. (O) Outlier and (Δ) extreme values.