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Does early EBV infection protect against IgE sensitization?

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Background: There is indirect evidence that an increased infectious burden is associated with a decreased prevalence of IgE-mediated allergy during childhood.
Objective: To determine whether there is a relation between the serostatus of 13 different viruses and parentally reported infections and IgE sensitization in 2-year-old children. To investigate whether there is an interaction between cytomegalovirus (CMV) and Epstein-Barr virus (EBV) in relation to IgE sensitization.
Methods: A total of 246 infants were followed prospectively to 2 years of age with clinical examinations, skin prick test, and specific IgE analyses and through analysis of seropositivity against adenovirus, influenza, parainfluenza, respiratory syncytial virus, CMV, EBV, herpes simplex virus, human herpesvirus 6, and varicella-zoster virus.
Results: There was some evidence that IgE sensitization (24%) tended to be more common among children who were seropositive against few compared with children who were seropositive against many viruses, but this was not statistically significant, and there was no consistent trend across the groups. IgE sensitization was statistically significantly less prevalent at 2 years of age among infants who were seropositive against EBV but not other viruses (adjusted odds ratio, 0.34; 95% CI, 0.14-0.86). The interaction of seropositivity against both CMV and EBV antibodies indicated a further reduction in the risk for IgE sensitization (adjusted odds ratio for interaction, 0.10; 95% CI, 0.01-0.92), indicating effect modification associated with seropositivity against CMV.
Conclusion: Our results indicate that acquisition of EBV infection during the first 2 years of life is associated with a reduced risk of IgE sensitization, and this effect is enhanced by CMV coinfection. (J Allergy Clin Immunol 2005;116: 438-44.)

Key words: *Childhood, CMV, EBV, IgE, infections, sensitization, serology, viral infections*

The increasing prevalence of allergic disease has become a major health problem in the industrialized parts of the world.[1] Epidemiological studies have shown that various markers of increased burden of infections are associated with a decreased prevalence of allergy and asthma during childhood.[2,3] Viral infections have been implicated in influencing IgE-mediated sensitization, but their exact role remains controversial.[3,4] It has been sugested that many viruses influence the differentiation of T cells, thus causing an imbalance between T_H1 and T_H2 immune responses.[5] Cytomegalovirus (CMV) and Epstein-Barr virus (EBV) are persistent viral infections, as demonstrated by frequent presence of the virus in saliva and urine of healthy individuals,[6] and may influence the immune system with respect to the development of allergy, as recently proposed by Sidorchuk et al.[7]

The aim of this study was therefore to elucidate the interplay between CMV and EBV in relation to IgE sensitization among children at 24 months of age. The study was also designed to investigate the association of IgE sensitization with infectious burden measured as seroprevalence against 13 different viruses, including CMV, EBV, and respiratory syncytial virus (RSV), as well as parentally reported infections.

METHODS

Subjects

Families who where expecting a child were asked by the midwife at the maternity ward whether they were interested in participating in the study. Only parents who reported they had a history of allergy in the mother, in both parents, or in neither parent were eligible. The parents provided a blood sample and underwent skin prick tests (SPTs). Only parents whose SPT results confirmed their positive or negative history of respiratory allergy to pollen and/or furred pets were invited to continue. When evaluated at 24 months of age, 246 children (126 boys and 120 girls) born to the selected parents participated in the study. One hundred two children had 2 allergic parents, 75 children had an allergic mother, and 69 children had no parental history of allergy. All infants were born full-term (>35 weeks of gestation) at hospitals in Stockholm and had birth weights within the normal range (data not shown). The socioeconomic status of the

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Abbreviations used
EBV: Epstein-Barr virus
CMV: Cytomegalovirus
HHV6: Human herpesvirus 6
HSV: Herpes simplex virus
kU_A: Kilo Units allergen-specific antibodies
RSV: Respiratory syncytial virus
SPT: Skin prick test
VZV: Varicella-zoster virus

families was estimated through the father's occupation grouped according to the classification used by Statistics Sweden. Demographic data are presented in Table I.

The study was approved by the Human Ethics Committee at Huddinge University Hospital, Stockholm (Dnr 75/97, 113/97), and the parents provided informed consent.

Clinical evaluation

The children were followed from birth to 2 years of age and were clinically evaluated at ages 6, 12, 18, and 24 months by 1 pediatrician (C. N.).

Skin prick testing

Skin prick tests against food and inhalant allergens were performed among the children at 24 months of age according to the manufacturer's recommendation (ALK, Copenhagen, Denmark). The SPT 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). SPTs were also performed for inhalant allergens: cat, dog, *Dermatophagoides farinae*, birch, and timothy (Soluprick 10 Histamine Equivalent Prick test). All parents were skin prick tested against the same inhalant allergens as the children but also against horse, rabbit, and mugwort. Histamine chloride (10 mg/mL) was the positive control and the allergen diluent the negative control. The SPT was considered positive if the wheal diameter was $\geq$3 mm after 15 minutes.

Parents' report of infections

During the observation period, the parents were asked to record every infection that their child had in a structured diary. This included symptoms—runny nose, cough, vomiting, diarrhea, fever—and a doctor's diagnosis where relevant. The diary consisted of sets of structured questions, 1 set for each illness. The parents filled in the form every time the child was ill, marking the correct squares with an *X* and recording the date of the illness. Parents confirmed the events recorded in the diary when they visited the outpatient clinic with the child at ages 6, 12, 18, and 24 months. In an attempt to record missed illnesses, at each visit the parents were asked, ''Has your child had any illness since your last visit?'' If additional illnesses were mentioned, they were added to the diary.

Blood sampling

Venous blood samples were collected when the children were 24 months old. Plasma was separated by centrifugation and stored at −70°C pending analysis.

Specific IgE

Circulating IgE antibodies against cow's milk, egg white, peanut, cod fish, soy bean, wheat, cat dander, dog dander, birch pollen, timothy pollen, and *Dermatophagoides farinae* were determined in plasma with Pharmacia CAP-FEIA (Pharmacia-Upjohn, Uppsala, Sweden). A positive test was defined as an IgE antibody level $\geq$0.35 kilo Units allergen-specific antibodies (kU_A) per liter.

Classification of the children

In accordance with Johansson et al,[8] the child was classified as IgE-sensitized if at least 1 SPT was positive ($\geq$3 mm) and/or if specific IgE against at least 1 of the selected allergens was $\geq 0.35 kU_A/L$. To optimize the classification in IgE-sensitized and non–IgE-sensitized children, the results of the *in vivo* and *in vitro* analysis were combined.

Viral infections/serological methods

The serostatus against 13 viruses was investigated: respiratory tract infections, including adenovirus, influenza (A/H1, A/H3, and influenza B), parainfluenza (types 1, 2, 3), and RSV; and herpesvirus, including CMV, EBV, herpes simplex virus (HSV), human herpesvirus 6 (HHV6), and varicella-zoster virus (VZV).

IgG against the EBV capsid antigen and HHV6 was determined according to previously published immunofluorescence assays.[9,10] A specific fluorescence in dilution of 1/20 was regarded as a sign of seropositivity.

For HSV, CMV, and VZV IgG ELISA with purified nuclear antigens from the respective viruses cultivated in human fetal lung fibroblasts were used.[11,12] The cutoff for seropositivity was an absorbance of >0.2 at a dilution of 1/100.

IgG antibodies against influenza A/H1, A/H3, and influenza B were determined with ELISAs by using recombinant influenza antigens.[13] IgG antibodies against parainfluenza (serotypes 1, 2, 3), RSV, and adenoviruses were measured by ELISA. The viruses were cultured to full cytopathogenic effect either in human fetal lung fibroblasts (RSV, adenovirus) or MA 104 cells (monkey kidney cells, parainfluenza) and prepared mainly by ultracentrifugation and sonification of clarified supernatants. For adenovirus, sonicated, infected cells were used. Preparations from the respective cell lines were used as control antigen in the assays using cell culture antigen. Optical densities above 0.3 after subtraction of control antigen activity were regarded as a sign of past infection for the respiratory viruses.

Statistics

Descriptive statistics were used to characterize the data. χ^2 Analysis and the Student *t* test (2-tailed) were used for comparison of IgE-sensitized and non–IgE-sensitized children where appropriate. The number of serologically verified infections was normally distributed (Fig 1), and the parentally reported infections were close to normally distributed, and they were divided into quarters defined by quartiles by using the statistical program SPSS 11.0 for Windows (SPSS Inc, Chicago, Ill). There was variation in the size of the groups as a result of characteristics of the distribution.

Odds ratios and 95% CIs were calculated for the development of IgE sensitization. Data were adjusted for background variables by using multiple logistic regression analysis. Adjustments were made for sex, parental allergy (none, single-heredity, or double-heredity), maternal age, parental smoking, furred pets at home, months of birth, older siblings, duration of breast-feeding, socioeconomic status, parentally reported infections, and seropositivity against viruses. All of the measures were modeled as series of binary dummy variables.

The interaction of seropositivity for CMV and EBV was investigated by using logistic regression, with adjustment for the main effects.

P values <.05 were considered statistically significant. The data were analyzed by using Stata 7.0 (Stata Corp, College Station, Tex), SPSS 11.0 for Windows, and the SAS System for Windows release 8.02 (SAS Institute, Cary, NC).

TABLE I. Data among infants with positive SPT and/or positive specific IgE and infants with negative SPT/specific IgE at 24 months of age

	Whole cohort	IgE-sensitized	Nonsensitized	OR; 95% CI	OR_{adj}; 95% CI
n (%)	246	59 (24.0)	187 (76.0)		
Sex					
Boy, n (%)	126 (51.2)	34 (27.0)	92 (73.0)	1	1
Girl, n (%)	120 (48.8)	25 (20.8)	95 (79.2)	0.71; 0.39-1.28	0.65; 0.32-1.31
Heredity					
Nonheredity; n (%)	69 (28.0)	11 (15.9)	58 (84.1)	1	1
Double-heredity; n (%)	102 (41.5)	32 (31.4)	70 (68.6)	2.41; 1.12-5.20	3.79; 1.50-9.60
Maternal heredity; n (%)	75 (30.5)	16 (21.3)	59 (78.7)	1.43; 0.61-3.34	2.29; 0.83-6.31
Maternal age at delivery					
Maternal age, mean (range)	31.4 (21-44)	31.5 (22-44)	31.3 (21-43)		
21-30 y, n (%)	103 (41.9)	22 (21.6)	81 (78.4)	1	1
31-44 y, n (%)	143 (58.1)	37 (25.9)	106 (74.1)	1.29; 0.70-2.35	1.42; 0.67-3.01
Month of birth					
Born April-September, n (%)	148 (60.2)	27 (18.2)	121 (81.8)	1	1
Born October-March, n (%)	98 (39.8)	32 (32.7)	66 (67.3)	2.17; 1.20-3.93	2.23; 1.10-4.52
Exposure					
Exclusive breast-feeding					
0 mo, n (%)	13 (5.3)	2 (15.4)	11 (84.6)	0.61; 0.13-2.89	0.91; 0.13-6.60
0.5-3.9 mo, n (%)	34 (13.8)	10 (29.4)	24 (70.6)	1.40; 0.62-3.19	3.11; 1.10-8.85
4-5 mo, n (%)*	166 (67.5)	38 (22.9)	128 (77.1)	1	1
5.1-10 mo, n (%)	33 (13.4)	9 (27.3)	24 (72.7)	1.26; 0.54-2.95	1.21; 0.45-3.21
Mothers smoking, n (%)	10 (4.1)	1 (1.7)	9 (4.8)	0.34; 0.04-2.75	0.32; 0.03-3.19
Fathers smoking, n (%)	19 (7.7)	7 (11.9)	12 (6.4)	1.96; 0.73-5.24	2.24; 0.68-7.34
Furred pets at home, n (%)	45 (18.3)	9 (15.3)	36 (19.3)	0.76; 0.34-1.68	0.74; 0.28-1.94
Number of older siblings					
0, n (%)	135 (54.9)	34 (25.2)	101 (74.8)	1	1
1, n (%)	81 (32.9)	24 (29.6)	57 (70.4)	1.25; 0.68-2.31	1.35; 0.65-2.80
>2, n (%)	30 (12.2)	1 (3.3)	29 (96.7)	0.10; 0.01-0.78	0.07; 0.01-0.65
Socioeconomic status†					
High, n (%)	141 (57.3)	34 (24.1)	107 (75.9)	1	1
Medium, n (%)	39 (15.9)	9 (23.1)	30 (76.9)	0.94; 0.41-2.19	0.97; 0.36-2.64
Low, n (%)	41 (16.7)	10 (24.4)	31 (75.6)	1.02; 0.45-2.28	1.35; 0.49-3.75
Studying, n (%)	11 (4.5)	4 (36.4)	7 (63.6)	1.80; 0.50-6.52	3.39; 0.67-17.06
Not specified, n (%)	14 (5.7)	2 (14.3)	12 (85.7)	0.52; 0.11-2.46	0.52; 0.09-3.14

OR, Odds ratio; OR_{adj}, adjusted odds ratio.
*The recommended time for exclusively breast-feeding is at least 4 months in Sweden.
†Grouped by the father's occupation according to Statistics Sweden (Swedish government for official statistics).

RESULTS

IgE sensitization

The children had an average age of 24.1 months (range, 22-29) at the 24-month evaluation. Fifty-nine (24%) children were classified as IgE-sensitized. The *in vitro* test (allergen-specific IgE in plasma) was positive in 52 (22%) infants, whereas 35 (14%) infants had at least 1 positive SPT against the selected food and inhalant allergens. The majority (n = 49; 83%) were sensitized against food allergens, and sensitization toward individual allergens was for milk, 13.4%; egg, 7.7%; peanut, 6.1%; dog, 4.9%; wheat, 4.1%; cat, 3.7%; birch, 3.7%; soy, 2.0%; fish, 1.2%; timothy, 0.8%; and mite, 0.8%.

There were no statistically significant differences in sex, having a furred pet at home, or having smoking parents between IgE-sensitized and nonsensitized children (Table I).

However, the nonsensitized children were statistically significantly more likely to have been born during the summer than sensitized children and more frequently had more than 1 sibling. The sensitized children were statistically significantly more likely to have 2 atopic parents. The statistically significant association with short duration of breast-feeding was observed only in the adjusted analyses, suggesting a complex set of associations or a chance finding.

IgE sensitization and the parental report of infections

The median number of parental reported infections was 13 (range, 4-24) during the first 24 months of life. For evaluation, the infants were divided into 4 groups defined by quartiles: 4 to 9, 10 to 13, 14 to 16, and 17 to 24 reported infections. The association with IgE sensitization

for each group was evaluated. The sensitized children were fewer in the group with 10 to 13 parentally reported infections, but there were no statistically significant difference between the groups, and the trend across the groups was not consistent (Table II).

IgE sensitization and the frequency of seropositivity

The frequency of seropositivity at 24 months of age against the selected viruses is presented in Table II. All children apart from 1 had detectable IgG antibodies against at least 1 of the viruses studied, and 1 child had detectable IgG against all 13. There were no significant associations between the number of parentally reported infections and the number of viruses identified through serology (data not shown).

The mean number of viruses identified through serology was 5 (Fig 1). The children were divided into 4 groups defined by quartiles. Fifty-five children had antibodies against 0 to 3 viruses, 95 children against 4 to 5 viruses, 42 against 6 viruses, and 54 against 7 to 13 viruses, respectively. There was some suggestion that children with fewer antibodies were more often sensitized than children with antibodies against many viruses, but this result was not statistical significant (Table II), and the pattern did not show a consistent trend across the groups.

IgE sensitization and seropositivity to individual viruses

Respiratory viruses. The association between presence of antibodies against the individual viruses and IgE sensitization is presented in Table II. There were no statistically significant associations between IgE sensitization and seropositivity against the 8 airborne viruses—adenovirus, influenza (A/H1, A/H3, and B), parainfluenza (types 1, 2, 3), and RSV. Sixty-three percent (154 children) were seropositive against RSV at 24 months of age, and among these, 21% (n = 33) were IgE-sensitized.

Herpesviruses. Seropositivity against the viruses belonging to the herpesvirus family—CMV, EBV, HSV, HHV6 and VZV—and IgE sensitization are also presented in Table II. Seronegativity against EBV was statistically significantly associated with IgE sensitization. Sixty-four children (26%) were seropositive against EBV. Among these, 8 (12 %) were sensitized. This was significantly less than in the EBV seronegative group (odds ratio, 0.37; 95% CI, 0.16-0.82). The association remained statistically significant after adjustment for all of the potential confounding factors (Table II).

Cytomegalovirus IgG antibodies were detectable in 96 (39%) children. Among these, 27 (28%) were IgE-sensitized. There were no statistically significant differences in the numbers of seropositivity or seronegativity against CMV when comparing the IgE sensitized and nonsensitized infants. However, seropositivity for EBV was more negatively associated with sensitization among subjects who were also seropositive for CMV. The odds ratio for

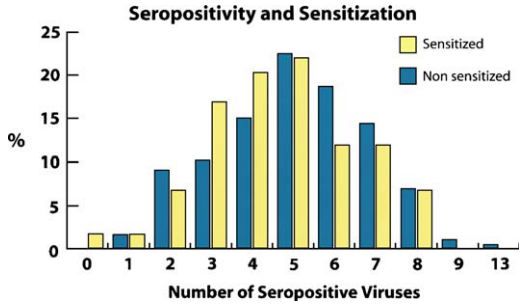

FIG 1. Frequency of seropositivity against the 13 selected viruses at 24 months of age in IgE sensitized* (n = 59) and nonsensitized (n = 187) infants. *At least 1 positive SPT ≥3 mm and/or at least 1 specific IgE against the selected allergens ≥0.35 kU_A/L.

IgE sensitization associated with seropositivity against EBV among children seronegative against CMV was 0.75 (95% CI, 0.30-1.91) but was reduced to 0.07 (95% CI, 0.01-0.57) among children who were seropositive against both viruses. Interaction testing revealed a statistically significant synergistic effect (effect modification), producing an odds ratio for the interaction of both viruses with IgE sensitization of 0.10 (95% CI, 0.01-0.92) after adjustment for the main effects.

We also observed an association with sensitization among subjects who were seropositive against CMV and seronegative against EBV. Among those seronegative against EBV (n = 182), 26 of 71 were sensitized among those infected with CMV, compared with 25 of 111 who were not infected with CMV. This produces an odds ratio of 1.99 (95% CI, 1.03-3.83), suggesting a modest increased risk associated with CMV infection in subjects who are seronegative for EBV.

The serostatus against the other herpesviruses was not significantly associated with IgE sensitivity.

DISCUSSION

The hypothesis that infectious diseases during early childhood may have a protective role against the development of allergy, the hygiene hypothesis, was raised in the late 1980s.[5]

In the current study, we did not find strong evidence of an association between IgE sensitization and the number of previous infections indicated either by parental report or by seropositivity. However, IgE sensitization was less prevalent at 2 years of age among infants who were seropositive against EBV. The combination of having both CMV and EBV antibodies was more strongly negatively associated with sensitization than would be predicted by the individual associations of EBV or CMV antibodies alone, indicative of an interactive effect.

The prevalence of EBV antibodies in our study is comparable with that of other industrialized countries.[14] Previous studies of EBV are contradictory in relation to development of atopy. Increased levels of antibodies against EBV were found in children 5 to 18 years old

TABLE II. Seropositivity against the investigated viruses and parentally reported infections in IgE-sensitized and nonsensitized children

	Whole cohort	IgE-sensitized	Nonsensitized	OR; 95% CI	OR_{adj}; 95% CI
n	246	59	187		
Parainfluenza 1, n (%)	70 (28.5)	16 (27.1)	54 (28.9)	0.92; 0.48-1.76	0.93; 0.46-1.86
Parainfluenza 2, n (%)	30 (12.2)	7 (11.9)	23 (12.3)	0.96; 0.39-2.36	1.14; 0.44-2.99
Parainfluenza 3, n (%)	166 (67.5)	42 (71.2)	124 (66.3)	1.26; 0.66-2.38	1.13; 0.58-2.21
Influenza Panama, n (%)	47 (19.1)	13 (22.0)	34 (18.2)	1.27; 0.62-2.61	1.32; 0.62-2.82
Influenza Texas, n (%)	30 (12.2)	6 (10.2)	24 (12.8)	0.76; 0.30-1.97	0.63; 0.23-1.76
Influenza Beijing, n (%)	92 (37.4)	16 (27.1)	76 (43.8)	0.54; 0.28-1.03	0.52; 0.26-1.01
Adenovirus, n (%)	200 (81.2)	46 (78.0)	154 (82.4)	0.76; 0.37-1.56	0.77; 0.36-1.63
RSV, n (%)	154 (62.6)	33 (55.9)	121 (64.7)	0.69; 0.38-1.26	0.71; 0.37-1.36
HHV6, n (%)	207 (84.2)	47 (79.7)	160 (85.6)	0.66; 0.31-1.40	0.66; 0.30-1.46
HSV, n (%)	24 (9.8)	6 (10.2)	18 (9.6)	1.06; 0.40-2.81	1.10; 0.40-3.02
CMV, n (%)	96 (39.0)	27 (45.8)	69 (36.9)	1.44; 0.80-2.60	1.20; 0.64-2.27
VZV, n (%)	45 (18.3)	8 (13.6)	37 (19.8)	0.64; 0.28-1.45	0.60; 0.25-1.42
EBV, n (%)	64 (26.0)	8 (13.6)	56 (29.9)	0.37; 0.16-0.82	0.34; 0.14-0.86
Frequency of seropositivity					
0-3 viruses, n (%)	55 (22.4)	16 (27.1)	39 (20.9)	1.57; 0.71-3.50	1.53; 0.59-3.97
4-5 viruses, n (%)	95 (38.6)	25 (42.4)	70 (37.4)	1.15; 0.47-2.84	0.78; 0.28-2.21
6 viruses, n (%)	42 (17.1)	7 (11.9)	35 (18.7)	1.02; 0.42-2.51	0.99; 0.35-2.80
7-13 viruses, n (%)	54 (22.0)	11 (18.6)	43 (23.0)	1	1
Parentally reported infections					
4-9 infections, n (%)	54 (22)	17 (28.8)	37 (19.8)	1.48; 0.64-3.46	1.40; 0.50-3.95
10-13 infections, n (%)	75 (30.5)	13 (22.0)	62 (33.2)	0.68; 0.29-1.61	0.57; 0.21-1.54
14-16 infections, n (%)	62 (25.2)	16 (27.1)	46 (24.6)	1.12; 0.48-2.61	1.11; 0.40-3.08
17-24 infections, n (%)	55 (22.3)	13 (22.0)	42 (22.5)	1	1

OR, Odds ratio; OR_{adj}, adjusted odds ratio.

with clinical signs of atopy compared with their nonatopic counterparts.[15] However, Calvani et al[14] reported a higher prevalence of atopy among EBV seronegative children in the age group 0 to 6 years, corroborating our results. A recent Swedish study (BAMSE) failed to demonstrate that the EBV serostatus in 4-year-old children correlated with IgE sensitization.[16] In developing countries, where EBV asymptomatically infects the majority of children before 3 years of age,[17] the prevalence of atopy has been reported to be lower than in industrialized countries.[18] In combination with our findings, these observations might indicate an age-dependent role of EBV, rather than EBV infection per se, in relation to IgE sensitization.

Cytomegalovirus becomes persistent after primary infection and seems to induce a T_H1 cytokines response.[19] CMV is frequently transmitted from mothers to infants during pregnancy, at delivery, or via breast milk.[20] The few studies published on the relation between CMV and allergies are inconclusive.[21] In the Swedish BAMSE study, no association was found between CMV seropositivity and IgE sensitization in 4-year-old children. However, among children with seropositivity against CMV and seronegativity against EBV, there was a positive association with sensitization to food allergens.[7] This led us to test the interaction of seropositivity against CMV and EBV in relation to IgE sensitization. This indicated effect modification such that the negative association of EBV seropositivity with sensitization was further enhanced in children who were also seropositive for CMV. The mechanism for this putative interaction is unknown. Several plausible explanations are possible, including the idea that IL-10 homologues present in the viruses might downregulate the antigen processing/presentation capacity of dendritic cells/macrophages and thereby switch off the host T-cell system, similar to the downregulation observed for T regulatory cells.[22,23] Alternately, both EBV and CMV can polyclonally activate B cells to produce antibodies with many different specificities and thereby hinder the capacity of allergens to cross-link the B-cell receptor as seen for helmintic infections.[24] Thus, these data and our results provide further support for the hypothesis that specific characteristics of EBV and/or CMV infection, rather than infection per se, might influence the risk of IgE sensitization.

Interestingly, RSV infection, according to serology, was not associated with IgE sensitization at 24 months of age. Previous studies have suggested that hospitalization because of RSV infection is linked with atopy and induction of IgE synthesis.[25] Discrepancies between different studies indicate that the vulnerability to RSV might be associated with a propensity for asthma and allergy, and not that RSV per se causes asthma. Again, the characteristics of infection may be important, and identification through hospitalization suggests more severe acute infection than the majority of children in our study would have had. Blanco-Quiros et al[26] reported that infants who developed severe RSV bronchiolitis had low levels of IL-12, a strong T_H1 inducer, in cord blood. We have recently published similar data showing low levels of IL-12 in cord blood of IgE sensitized infants at 24 months of age.[27]

These observations might indicate an interaction among cytokines, RSV, and sensitization during early infancy.

There was some indication that the numbers of serologically verified viral infections were inversely correlated to IgE sensitization, but there was no consistent trend across the groups and no statistically significant association. The number of parentally reported infections and seropositivity against the selected viruses did not correlate. This is not entirely surprising, because many viruses, eg, rhinovirus and corona virus, which are proposed to be the major causes of upper respiratory infections in infants, were not included in our analysis.[28] There is not always a relation between viral infections and diseases, because asymptomatic infections are common. The suggestion of an inverse association between the frequencies of parentally reported and serologically verified viral infections with sensitization is in agreement with previous publications,[29,30] although these authors have mainly studied clinical signs of allergy. It is possible that the suggested protective effect of infections continues to influence the nascent immune system beyond age 2 years, and our follow-up was conducted at too young an age to observe the protective effect against allergic disease.

Previous studies have shown mainly indirect evidence for the importance of infections for the development of allergy,[3,5,29] and these studies have often been retrospective. The strength of our study is that the atopic status of the parents was characterized, and information on infections was collected prospectively by using diaries. Importantly, objective serological measurements against antiviral antibodies were made, and the results were adjusted for factors that might bias the evaluation.

However, we recognize some limitations. The study population was selected and not population-based. Because we selected children with different family histories of allergy, we believe that the group of children in our study is fairly comparable with children in the general population. Furthermore, the exact sensitivity for seropositivity in some of the assays used to evaluate the respiratory infections is not fully known because they have been used mainly to detect ongoing infections, so the rate of seropositivity is somewhat underestimated, but this should not introduce bias. The optimal approach would have been to perform neutralization assays, but serum samples from infants are inevitably insufficient for tests using low serum dilutions. In contrast, the sensitivity and specificity for seropositivity of the assays used for the herpesvirus infections have been proven reliable, as best demonstrated by the correlation between initial serostatus and clinical outcome found in transplant recipients.[31] It could also be argued that analyzing 13 different viruses could produce some associations with IgE sensitization by chance. However, because an association specifically with EBV was evaluated as a result of an a priori hypothesis based on the results of previous studies,[14] the associations reported for EBV are unlikely to be caused by chance.

In summary, our results indicate that an EBV infection during the first 2 years of life is associated with a reduced risk of IgE sensitization. Some patterns of infection may confer greater protection, such as EBV infection in the age-defined window of susceptibility with possible modification through interaction with CMV. Development of more precise measures of patterns of acute infection and their immunological sequel will assist in our understanding of how early in life viral infections are implicated in the etiology of IgE sensitization.

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