

Early-life EBV infection protects against persistent IgE sensitization

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Background: Infection with 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.

Objective: 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 5 years of age with clinical examinations, skin prick testing, specific IgE analyses, and determination of serostatus against EBV.

Results: After analysis of the children's EBV serostatus, we found that 5-year-olds who were infected with EBV before the age of 2 years were at a significantly lower risk of being persistently IgE-sensitized—that is, sensitized at both 2 and 5 years of age (adjusted odds ratio, 0.34; 95% CI, 0.12-0.94). In contrast, contraction of EBV after 2 years of age was highly associated with late-onset IgE sensitization (adjusted odds ratio, 4.64; 95% CI, 1.57-13.69).

Persistently sensitized 5-year-olds had higher specific-IgE levels than children with late-onset IgE sensitization ($P < .01$).

Conclusion: Our data support the value of early-life microbial exposure for protection against the development of IgE sensitization and underscore the proximate postnatal years as an important period during which EBV could contribute to an allergo-protective immune profile. (J Allergy Clin Immunol 2009;123:1009-1015.)

Key words: Epstein-Barr virus, seronegative, seropositive, age-dependent dual roles of infection, polyclonal stimulatory effect, persistent IgE sensitization

Abbreviations used

OR: Odds ratio

OR_{adj}: Adjusted odds ratio

SPT: Skin prick test

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 factors.¹ An altered overall spectrum of infections may set the stage for an imbalanced T_H1/T_H2 immune profile, which could contribute to the development of allergies.^{2,3}

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

Infections with EBV and cytomegalovirus have previously been implicated in influencing allergic disorders, but their precise role remains controversial. Some studies have suggested allergy-protective^{8,9} or allergy-promoting¹⁰⁻¹² effects, whereas others could not demonstrate a clear role for EBV or cytomegalovirus 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 2-year-old infants.⁹ We also observed that seropositivity with cytomegalovirus in the absence of EBV tended to associate with a higher risk of IgE sensitization. This suggests that infection with EBV during the first 2 years of life may protect from allergy. We have also recently demonstrated that both EBV and cytomegalovirus might influence early-life development toward diverse immune profiles.¹⁵

The current study aimed to investigate further 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 5 years of age. After analysis of the children's EBV and

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cytomegalovirus serostatus, we demonstrate that early-life EBV infection significantly relates to protection against persistent IgE sensitization. Further, in sharp contrast with the group that was infected before the age of 2 years, those subjects who contracted EBV after the age of 2 years were at a markedly higher risk of becoming IgE-sensitized later in childhood.

METHODS

Study population and determination of serostatus against EBV and cytomegalovirus

This study includes 219 children (Table I) born between 1997 and 2000 in Stockholm who are part of a prospective study cohort ($n = 281$). One third of the children had 2 parents with allergy, another third had a mother with allergy, 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 published.⁹ 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 and 4.8 kg with a mean of 3.6. The presence of plasma IgG against EBV capsid antigen 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 1.¹⁷ IgG seropositivity to cytomegalovirus was determined by ELISA using purified nuclear antigens from cytomegalovirus cultivated in human fetal lung fibroblasts,¹⁸ where samples with an absorbance above 0.2 at a dilution of 1/100 were considered positive. Because 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 5 years are as follows: both EBV and cytomegalovirus serostatus were determined in 192 of 219 five-year-olds, and data for cytomegalovirus serostatus were determined in 195 (3 additional subjects) 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 (Soluprick, ALK, Copenhagen, Denmark). The SPT at 2 and 5 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 Histamine Equivalent in SPT, HEP). In addition to these allergens, subjects were tested for rabbit at the age of 5 years (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 controls, 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 1 pediatrician (C.N.) at age 6, 12, 18, 24, and 60 months. In accordance with Johansson et al,¹⁹ the child was classified as IgE-sensitized if 1 or more SPTs were positive (≥ 3 mm) and/or if specific IgE against 1 or more of the selected allergens was ≥ 0.35 kU_A/L. A total of 225 children (80%) were eligible for evaluation of IgE sensitization at both 2 and 5 years of age. Among these, 6 children were excluded because the results

of the EBV analyses were indeterminate despite repeated examination or because of insufficiency of plasma volumes for performance of additional analyses. The remaining 219 subjects were classified according to the presence or absence of IgE sensitization as follows: nonsensitized (IgE-sensitized at neither 2 nor 5 years of age), $n = 129$ (59%); transiently sensitized (IgE-sensitized at 2 but not 5 years of age), $n = 7$ (3%); persistently sensitized (IgE-sensitized at both 2 and 5 years of age), $n = 48$ (22%); and late-sensitized (IgE-sensitized at 5 but not 2 years of age), $n = 35$ (16%; Table I).

Statistical methods

Odds ratio (OR) estimates and corresponding 95% CIs for development of IgE sensitization were obtained by logistic regression. Data were adjusted for the 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. The Mann-Whitney *U* test was used to assess differences in IgE levels between sample groups. The Fisher exact test was applied to evaluate differences between the transiently sensitized and nonsensitized group and to test differences in susceptibility to EBV or cytomegalovirus infection between sensitized and nonsensitized subjects. Statistical analyses were performed at the Division of Mathematical Statistics, Stockholm University. *P* values below .05 were considered statistically significant.

RESULTS

IgE sensitization among 5-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, whereas 46 children (96%) had a positive test for circulating IgE against 1 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 ($P < .01$; Fig 1). No significant differences were seen among nonsensitized, persistently sensitized, and late-sensitized children with regard to maternal age, birth weight, gestation week, or maternal or paternal smoking (data not shown). We observed, however, that IgE-sensitized children were significantly more likely to have 2 parents with allergy and that the group of persistently sensitized children was more likely to be born during the winter than late-sensitized and nonsensitized children. Further, delivery by cesarean section was more common among late-sensitized children (Table I). The group of transiently IgE-sensitized children ($n = 7$) did not differ from the nonsensitized group regarding any of the investigated demographic parameters (data not shown).

EBV and cytomegalovirus seroprevalence among the 2-year-olds and 5-year-olds

Data for the whole cohort showed that among the 219 two-year-olds, 109 (50%) children were seronegative for both EBV and cytomegalovirus, 25 (11%) were seropositive for EBV alone, and 66 (30%) were only cytomegalovirus seropositive. The remaining 19 (9%) were seropositive for both herpesviruses. Corresponding results for 5-year-olds ($n = 192$) demonstrated that 30 (16%) children were seronegative for both EBV and cytomegalovirus, 20 (10%) were seropositive for EBV alone, 89 (46%) were only cytomegalovirus-seropositive, and the remaining 53 (28%) were seropositive for both herpesviruses (data not shown).

TABLE I. 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
No. (%)	219	129 (58.9)	48 (21.9)	35 (16.0)				
Sex								
Boy, no. (%)	114 (52.1)	68 (52.7)	27 (56.3)	15 (42.9)	1	1	1	1
Girl, no. (%)	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								
Nonheredity, no. (%)	62 (28.3)	47 (36.4)	8 (16.7)	6 (17.1)	1	1	1	1
Double heredity, no. (%)	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, no. (%)	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, no. (%)	183 (83.6)	109 (84.5)	42 (87.5)	26 (74.3)	1	1	1	1
Cesarean, no. (%)	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, no. (%)	136 (62.1)	88 (68.2)	22 (45.8)	22 (62.9)	1	1	1	1
October-March, no. (%)	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 mo, no. (%)	10 (4.6)	6 (4.7)	2 (4.2)	2 (5.7)	1	1	1	1
0.5-3.9 mo, no. (%)	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 mo, no. (%)	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 mo, no. (%)	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, no. (%)	27 (12.3)	12 (9.3)	9 (18.8)	6 (17.1)	1	1	1	1
Yes, no. (%)	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 (mo), median (range)	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, no. (%)	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
No. 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

OR_{adj} 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. Boldface indicates statistically significant.

*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 nonsensitized and persistently IgE-sensitized.

||Comparison between nonsensitized and late IgE-sensitized.

¶Analysis based on continuous variable because of the presence of 0 value.

Relation between IgE sensitization and EBV and cytomegalovirus infection during early childhood

We examined the frequency of EBV and cytomegalovirus seropositivity in relation to IgE-sensitization during infancy and childhood (Table II). The transiently sensitized group was excluded from these analyses because it contained too few subjects (n = 7) for a reliable statistical outcome. Because the number of subjects with determined serostatus differed between the 2-year-olds and 5-year-olds, Table II was divided into a section where relation between serostatus at age 2 years and persistent IgE sensitization was evaluated and a section where serostatus at 2 years or between 2 and 5 years of age was related to late IgE sensitization. We found that the 5-year-old children who were EBV-seropositive at 2 years of age were at a decreased risk of being persistently IgE-sensitized (adjusted

OR [OR_{adj}], 0.34; 95% CI, 0.12-0.94). This association was not observed for cytomegalovirus infection, which rather tended to be associated with an increased risk (OR_{adj}, 1.66; 95% CI, 0.84-3.31). In sharp contrast with EBV infection before the age of 2 years, EBV seropositivity acquired between the ages of 2 and 5 years was related to a significantly higher risk of having developed IgE sensitization at 5 years of age (late IgE-sensitized; OR_{adj}, 4.64; 95% CI, 1.57-13.69). No such association was observed for cytomegalovirus infection. The frequency of early EBV infection—that is, seropositivity at 2 years of age—did not differ between late-sensitized and nonsensitized 5-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

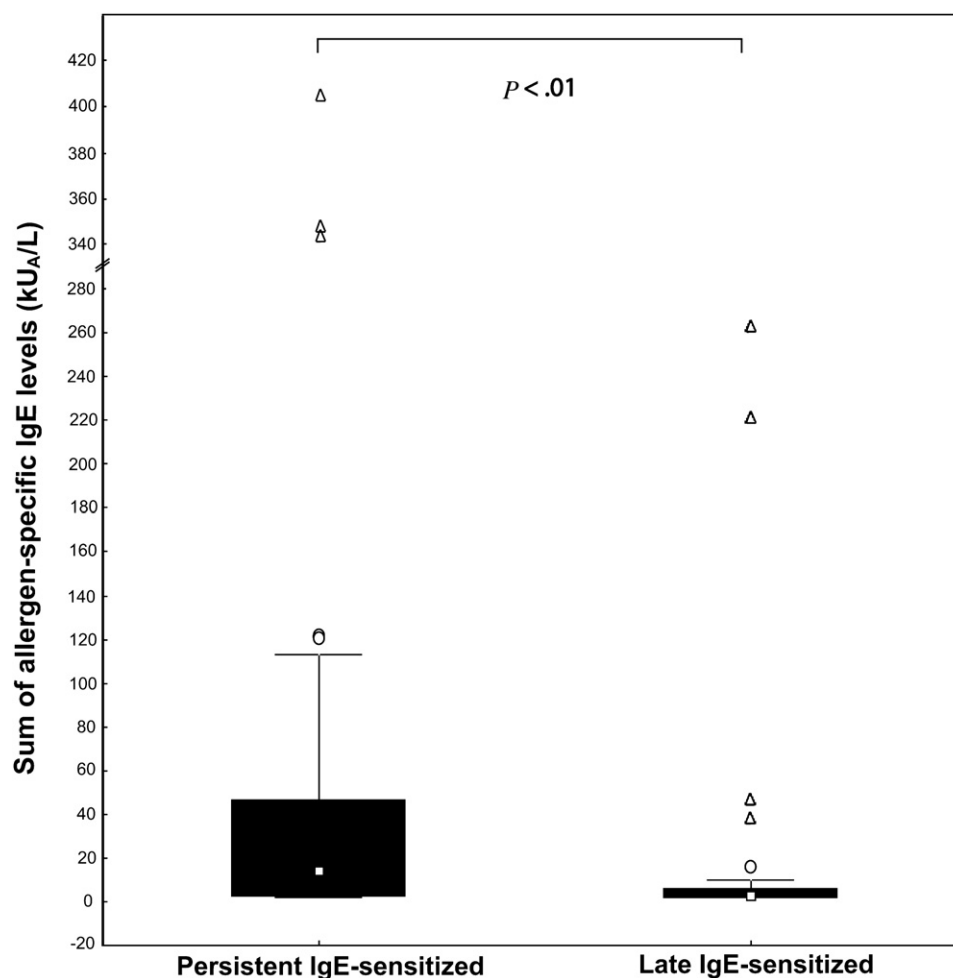


FIG 1. Allergen-specific IgE levels in 5-year-old persistently IgE-sensitized ($n = 46$) or late IgE-sensitized ($n = 35$) subjects. Boxes cover values between 25th and 75th percentiles, the central square within the boxes represents the median, and whiskers span to nonoutlier values. Outlier (O) and extreme (Δ) values.

1 group and compared with the nonsensitized children (data not shown).

To evaluate whether the exclusion of these children influenced the findings, we regrouped the transiently sensitized and the persistently sensitized subjects into 1 group, given that the children in both groups were IgE-sensitized at 2 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, data not shown).

Relation between IgE sensitization and susceptibility to EBV and cytomegalovirus

We also addressed whether susceptibility to EBV or cytomegalovirus is altered in IgE-sensitized subjects. To this end, we excluded 2-year-olds who were seropositive for both EBV and cytomegalovirus, divided the remaining children into IgE-sensitized or nonsensitized, and analyzed the frequency of EBV and/or cytomegalovirus seropositivity at 5 years of age. There were no differences in the proportion of subjects who seroconverted for EBV and/or cytomegalovirus between the IgE-sensitized and nonsensitized group (Table III), suggesting that acquisition of

EBV and cytomegalovirus 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 period during infancy, because infection with EBV after 2 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 because 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 nonpathogenic microorganisms during the earliest periods of life for proper shaping of the immune system away

TABLE II. Frequency of EBV and cytomegalovirus seropositivity among IgE sensitized and nonsensitized children

Serostatus at 2 y of age	Total	Nonsensitized at 5 y	Persistent IgE-sensitized at 5 y	OR; 95% CI	OR _{adj} ; 95% CI
No.	212	164	48		
EBV+, no. (%)	43 (20.3)	38 (23.2)	5 (10.4)	0.39; 0.14-1.04	0.34; 0.12-0.94
CMV+, no. (%)	83 (39.2)	61 (37.2)	22 (45.8)	1.43; 0.75-2.74	1.66; 0.84-3.31
Serostatus at 5 y of age*	Total	Nonsensitized at 5 y	Late IgE-sensitized at 5 y	OR; 95% CI	OR _{adj} ; 95% CI
EBV serostatus determined, no.	146	115	31		
EBV+ at the age of 2 y, no. (%)	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 y, no. (%)	27 (18.5)	17 (14.8)	10 (32.3)	3.07; 1.17-8.08	4.64; 1.57-13.69
CMV serostatus determined, no.	147	115	32		
CMV+ at the age of 2 y, no. (%)	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 y, no. (%)	57 (38.8)	46 (40.0)	11 (34.4)	0.65; 0.24-1.72	0.49; 0.17-1.45

CMV, Cytomegalovirus.

The results for the transiently IgE-sensitized children (n = 7) are not included in the serostatus at 2 and 5 years of age. OR_{adj} for parental allergy. Boldface indicates statistically significant.

*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 III. Relation between IgE-sensitization and susceptibility to EBV and cytomegalovirus

Antiviral serostatus at 2 y of age		Antiviral serostatus at 5 y of age		Non-IgE-sensitized at 2 y of age (non-IgE-sensitized and late IgE-sensitized)	IgE-sensitized at 2 y 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 = .53

CMV, Cytomegalovirus.

from the allergic phenotype.²² Our data indicate that early-life EBV infection may contribute to this immunomodulation and suggest that examination of defined age groups might overcome contradictions between previous reports on EBV's relation to allergy. Calvani et al⁸ observed that EBV infection during early childhood (especially <29 months) was related to protection against atopy, as opposed to primary EBV infection in older children. In the study by Sidorchuck et al,¹³ in which no association could be demonstrated, 4-year-old children were investigated, which in relation to our findings would be children exposed to both the protective effect before 2 years and the aggravating effect thereafter. In studies in which EBV infection has been pointed out as a risk factor in atopy, the investigated subjects were either between 5 and 18 years old¹⁰ or adults,¹¹ 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. Primary, symptomatic EBV infection is accompanied by a vigorous T-cell response and production of a multitude of antibodies,⁵ but the strength of such infection in asymptomatic children is unknown. However, elevated levels of both T_H1 and T_H2 cytokines have previously been demonstrated in 2-year-old EBV-seropositive infants,¹⁵ 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, whereas these are usually absent in asymptomatic infants with primary EBV infection.²³ Absence of B cells primed against the heterophile antigens because of 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 2 years 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 systems.²⁴ EBV, presumably in addition to other suggested microbial candidates such as intestinal bacteria,²⁵ might represent 1 such stimulatory agent. Indeed, the majority of children below 3 years of age are infected with EBV in developing countries,⁵ where the occurrence of allergic disorders is uncommon.²⁶ However, the asthma prevalence in those countries seems to be rising now, and increased industrialization has been proposed as a

cause.²⁷ Other viral agents have been discussed in relation to allergy, both as protective and as risk factors.²⁸ For instance, hepatitis A has been shown to correlate inversely to the prevalence of atopic diseases, and measles has been suggested to protect against IgE sensitization, but the results are contradictory.²⁹ In the case of respiratory syncytial virus infection, it appears that infants who require hospital care have a higher prevalence of asthma and allergy than children without severe symptoms.³⁰ Our data here do not support a risk modulation by cytomegalovirus, which is in agreement with previous observations.^{9,13} Collectively, these observations suggest that not only viral infections *per se*, but also 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 2-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 natural killer cells,³¹ whereas T-cell cytokine responses tend to be enhanced.¹⁵ 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 4 sensitization groups, and the transiently sensitized group 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 because regrouping the transient group with the persistent group did not alter the outcome of our findings, this is unlikely. Further, the seroprevalence of EBV (38%) among 5-year-olds in this cohort was lower than described for a previous cohort of 2561 Swedish children from the whole Stockholm region, in which 53% were EBV seropositive at the age of 4 years.¹³ Our smaller cohort size and the fact that all participants were recruited from a limited part of Stockholm might be possible explanations for the discrepancy. Because 2 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 to examine not EBV seroprevalence but the relation between infection with EBV and cytomegalovirus and IgE sensitization.

Findings from this study support a protective role for EBV infection before 2 years of age in relation to persistent IgE sensitization in childhood. Whether this group displays a differential atopic phenotype later in life compared with the late-onset group in our cohort remains to be determined; a 10-year follow-up is ongoing.

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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 1 such stimulatory agent.

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