

Epstein-Barr virus infection is not associated with development of allergy in children

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Background. Differences in concentrations of antibodies to Epstein-Barr virus (EBV) in atopic and nonatopic children have been observed, suggesting that EBV infection may play a role in allergic diseases.

Aim. To assess the association between EBV infection and atopy in Swedish children at 4 years of age.

Materials and methods. Were studied 2561 children born in 1994 through 1996 in Stockholm, Sweden. The children were enrolled in a prospective birth cohort study focusing on the relation of exposure to various environmental and life style factors during early childhood and development of atopy. Blood samples were obtained when the children were ~4 years old, and immunoglobulin G to EBV was determined by indirect immunofluorescence. The relationship between the seroprevalence to EBV and various allergic disorders was assessed, with the use of logistic regression analysis to account for other risk factors.

Results. Totally 1347 of 2561 (52%) children were EBV-seropositive. Associations between EBV seropositivity and the occurrence of asthma [adjusted odds ratio (OR_{adj}), 1.10; 95% confidence interval (95% CI) 0.81 to 1.49] or suspected allergic rhinitis (OR_{adj} 0.97; 95% CI 0.76 to 1.25) were not apparent. In children whose mothers were up to 25 years old, a higher EBV seroprevalence was observed than in children of older mothers (OR_{adj} 1.34; 95% CI 1.04 to 1.71). Also in children whose mothers smoked, the seroprevalence was

higher than in children of nonsmokers (OR_{adj} 1.29; 95% CI 1.02 to 1.63).

Conclusions. The study does not support the hypothesis that EBV infection in early childhood plays an important role in the pathogenesis of allergic diseases in children.

INTRODUCTION

The increasing prevalence of allergic diseases in children has become a major worldwide public health problem in recent decades.^{1,2} Changes in exposure to environmental factors, including infections, have been proposed as tentative explanations for the rise in prevalence of atopic diseases.^{3,4} Atopic diseases are characterized by dominant T helper (Th) 2 mechanisms and the production of IgE to common environmental allergens.⁵ Certain viral infections during infancy have been implicated as potentially responsible for the development of the asthmatic phenotype.^{6,7} For example respiratory syncytial virus (RSV) has been frequently observed to be associated with asthma in children.^{8,9} In addition some herpesviruses may be of interest. These viruses are ubiquitous pathogens, but primary infection is often delayed in developed countries. They establish life-long latency and are frequently reactivated. Herpesviruses may affect the immune system by increasing the Th1 or Th2 immune response.^{10–14}

Previous studies of the relation between Epstein-Barr virus (EBV) infection and subsequent development of an asthmatic phenotype have provided inconsistent results.^{15–18} Infection of humans with EBV usually occurs by contact with oral secretions. In industrialized countries virtually everyone has become seropositive by the age of 25 to 30 years, whereas seroconversion occurs earlier in developing countries.¹⁹ The virus replicates in cells in the oropharynx, and nearly all seropositive persons shed the virus in the saliva.²⁰ Infants become susceptible to EBV as soon as maternal antibody protection disappears. EBV induces B cell proliferation as well as polyclonal antibody proliferation,¹³ and primary EBV infection may result in manifestations of atopic diseases.²¹ Elevated antibodies to EBV have been demonstrated in atopic children and adults than in nonatopic individuals in some studies,^{15–17} but whether this is a result of increased

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virus replication associated with immune activation caused by the allergic reaction or a causal relationship between EBV infection and allergy has been difficult to clarify. In contrast a recent epidemiologic study indicates that EBV infection may protect against allergy in young children, but the association was reversed in older children.¹⁸ Also the high EBV seroprevalence among very young children in developing countries,^{22–24} where allergy is rare, could indicate a protective role for early EBV infection in the development of allergy.

In this study the seroprevalence to EBV and its relation to allergic diseases and sensitization in children at the age of ~4 years were examined. Swedish children who participated in a large, prospective birth cohort survey were studied [BAMSE (B = children (*barn*), A = allergy, M = environment (*miljö*), S = Stockholm, E = epidemiologic survey)].^{25–27}

MATERIALS AND METHODS

Study subjects. Children from the BAMSE prospective birth cohort were investigated. This cohort includes 4089 infants (2065 boys and 2024 girls) born from February 11, 1994 until November 22, 1996. The study design, description of the study cohort and procedures of data collection were presented previously.^{25–27} The parents of the included families answered questionnaires concerning various environmental factors as well as symptoms and health outcomes during the first years of life. The median ages of the children when parents returned different questionnaires from birth until 4 years of age are given in Table 1. The first questionnaire (Q₀) focused mainly on the parental history of allergic diseases and on various exposures. Subsequent questionnaires focused on symptoms of atopy in the children and were answered when the children were ~1 (Q₁), 2 (Q₂) and 4 (Q₄) years old.

The children whose parents answered Q₄ were also invited for a clinical examination and collection of blood samples. For different reasons 1128 children were not sampled. Among these 86 had moved out of Stockholm, and 291 families did not respond to the invitation for unknown reasons. It was not possible to draw blood from 350 children who took part in the

clinical examination; because of ethical reasons only one “try” was allowed. Another 401 families refused blood sampling. Thirty-three serum samples were excluded from the study of EBV antibodies because the volume of serum was not sufficient. Furthermore 20 children were excluded from the final analysis, because the results of the EBV analyses were indeterminate despite repeated examination. Thus 2561 of 3742 (68.4%) of the children whose parents answered Q₄ were included in the final study group (Table 1). Not all the parents came to the clinic for a clinical examination when their child was exactly 48 months, although most of the children were <5 years of age (99.8%).

In the BAMSE study, at 4 years of age asthma was defined as at least four episodes of wheezing during the last 12 months or at least one episode during the same period together with treatment of inhaled corticosteroids without an ongoing cold. Suspected allergic rhinitis was defined as runny, itchy or blocked nose during the last 12 months without an ongoing cold. The parents were asked for the occurrence of respiratory diseases apart from asthma, such as pneumonia, bronchitis and also RSV infection, all diagnosed by a doctor. The method used by the doctor to establish the diagnoses was not specifically requested.

Parents were asked about pneumonia and bronchitis in both Q₁ and Q₂. The questions were: “Has your child ever had a doctor’s diagnosis of pneumonia?” And also, “Has your child ever had a doctor’s diagnosis of bronchitis?” In Q₄ they were also asked: “Has your child had a doctor’s diagnosis of pneumonia after 2 years of age?” and “Has your child had a doctor’s diagnosis of bronchitis after 2 years of age?” Parents were asked about RSV infection only in Q₁. The question was: “Has your child had a doctor’s diagnosis of RSV infection?”

Children were classified into three categories for evaluation of allergic disease heredity: (1) children, who did not have any parent with allergic diseases; and children with (2) one or (3) both parents having ever been allergic. Maternal smoking was subdivided according to period: smoking (1) during pregnancy only and (2) at any time after birth of the child. Children were classified into two groups according to maternal age at the birth of the child: older than 25 years of age (the reference group); and mothers at 25 years of age and younger. Furthermore the children were subdivided into three categories related to breast-feeding: children who had never been breast-fed; those who had been breast-fed for <4 months; and children who had been breast-fed for 4 months and more. The variable “parental educational level” contained three categories: low level (one or both parents had passed compulsory school, equivalent to primary and lower secondary education or 2 years of upper secondary education); middle level (one or both parents had up to 3 years of upper secondary education, qualifying for college or

TABLE 1. Number of children followed in the prospective BAMSE birth cohort and participation at different stages of data collection

Data/Material Available	Median Age	No. of Participants*
Questionnaire 0	2 mo	4089 (100)†
Questionnaire 1	1 yr	3925 (95.9)
Questionnaire 2	2 yr	3843 (93.9)
Questionnaire 4	4 yr	3742 (91.5)
Blood samples	4.3 yr	2614 (63.9)
Serology (present study group)		2561 (62.6)

* See text for detailed description of reasons for nonparticipation.

† Numbers in parentheses, percent.

university studies); and high level (one or both parents held a college or university degree).

Serologic methods. IgG antibodies to the EBV capsid antigen (VCA) remain lifelong after the primary infection, and for determination of the EBV seroprevalence these antibodies were investigated by indirect immunofluorescence assay (IIF), according to previous publications.^{28–30}

Repeated analyses to confirm the validity of the serologic results were performed for selected samples. All the samples examined in 6 of 20 IIF assay sets, showing unusually high or low titers of seropositivity, and 5 positive and 5 negative samples from each remaining set of examination were chosen. The validity of the IIF assay was thus assessed for a total of 709 samples with the use of the kappa statistic and showed an agreement of 0.938.³¹

IgE antibodies to the most common airborne allergens were determined with Phadiatop [a mixture of mite (*Dermatophagoides pteronyssinus*), cat, dog, horse, timothy, birch, weed and mould], and antibodies to the most common food allergens determined with fx5 (a mixture of milk, fish, eggs, wheat, peanut and soya bean), Pharmacia CAP System (Pharmacia Diagnostics AB, Uppsala, Sweden) according to instructions from

the manufacturer. A positive result was defined as a concentration ≥ 0.35 kilounit/l.

Statistical methods. To assess the role of EBV infection for development of atopy, children were divided into groups depending on EBV serostatus. Odds ratio (OR) estimates and corresponding 95% confidence intervals (95% CI), for asthma, suspected allergic rhinitis, signs of sensitization (common and specific allergens) and respiratory tract infections (pneumonia, bronchitis and RSV infection) were obtained with logistic regression, adjusting for the potential risk factors for allergic diseases (gender, allergic heredity, maternal age, maternal smoking, duration of breastfeeding and parental educational level). The statistical package STATA 7.0 was used for analyses.³²

RESULTS

The distribution of socioeconomic characteristics, allergic diseases and respiratory tract infections among the 2561 participants were compared with those 1181 who were also invited, unless no data from blood samples were available (Table 2). There was a higher prevalence of bronchitis among the participants than among the nonparticipants, and the similar tendency

TABLE 2. Socioeconomic characteristics and occurrence of allergic outcomes and respiratory pathology in 1181 “nonparticipants” from the prospective BAMSE birth cohort in comparison with 2561 children in the study population

Risk Factors and Outcomes	Nonparticipants (N = 1181)	Study Population (N = 2561)	OR
Maternal smoking			
Never after birth of child	946 (80.1)*	2135 (83.4)*	1.0
At any time after birth of child	192 (16.3)	374 (14.6)	0.86 (0.71–1.04)†
Unknown‡	43 (3.6)	52 (2.0)	
Educational level of the parents			
High§	636 (53.9)	1347 (52.6)	1.0
Middle¶	302 (25.6)	699 (27.3)	1.09 (0.93–1.28)
Low	237 (20.0)	503 (19.6)	1.01 (0.84–1.20)
Unknown‡	6 (0.5)	12 (0.5)	
Allergic diseases			
Asthma			
No	1105 (93.6)	2352 (91.8)	1.0
Yes	70 (5.9)	190 (7.4)	1.28 (0.96–1.69)
Unknown‡	6 (0.5)	19 (0.7)	
Suspected allergic rhinitis			
No	1055 (89.3)	2230 (87.1)	1.0
Yes	117 (9.9)	296 (11.6)	1.19 (0.95–1.50)
Unknown‡	9 (0.8)	35 (1.4)	
Respiratory tract infections:			
Pneumonia			
Never	1101 (93.2)	2446 (95.5)	1.0
Ever	22 (1.9)	45 (1.8)	0.92 (0.55–1.54)
Unknown‡	58 (4.9)	70 (2.7)	
Bronchitis			
Never	1048 (88.7)	2269 (88.6)	1.0
Ever	70 (5.9)	204 (8.0)	1.35 (1.02–1.78)
Unknown	63 (5.4)	88 (3.4)	
RSV infection			
Never	1102 (93.3)	2413 (94.2)	1.0
Ever	46 (3.9)	107 (4.2)	1.06 (0.75–1.51)
Unknown‡	33 (2.8)	41 (1.6)	

* Numbers in parentheses, percent.

† Numbers in parentheses, 95% CI.

‡ Subjects answered questionnaires but no answer was given to this particular item.

§ One or both parents had held a college or university degree.

¶ One or both parents had up to 3 years of upper secondary education (qualifying for college or university studies).

|| One or both parents had passed compulsory school (equivalent to primary and lower secondary education) or 2 years of upper secondary education.

was observed for asthma and suspected allergic rhinitis.

The total number of EBV seropositives was 1347 of 2561 (52.6%). The relation between EBV seropositivity and different risk factors for the development of allergy is presented in Table 3. Maternal smoking after birth of the child was associated with a higher prevalence of EBV seropositivity [adjusted OR (OR_{adj}), 1.29; 95% CI 1.02 to 1.63]. In the group of children whose mothers were 25 years of age or younger, EBV seropositivity was more prevalent than in children whose mothers were older than 25 years of age (OR_{adj} 1.34; 95% CI 1.04 to 1.71). Additional adjustment for age at the time for blood sampling did not affect the results.

Table 4 shows the association between EBV seropositivity and allergy, sensitization and respiratory tract infections. No clear association was found between the seroprevalence to EBV and asthma (OR_{adj} 1.10; 95% CI 0.81 to 1.49) or symptoms of suspected allergic rhinitis (OR_{adj} 0.97; 95% CI 0.76 to 1.25). Neither was there any statistically significant association between seropositivity to EBV and occurrence of respiratory infections (pneumonia and bronchitis) or RSV infection. Furthermore no significant relation was seen between EBV serostatus and prevalence of allergen-specific IgE to common airborne or food allergens (OR_{adj} 0.90 and

95% CI 0.71 to 1.10; and OR_{adj} 0.84 and 95% CI 0.69 to 1.08), respectively.

DISCUSSION

The prevalence of EBV infection of ~50% in the children of the present study is in line with previous epidemiologic studies in Sweden and in other developed countries.^{18, 19, 33, 34} Except for smoking and young maternal age, there was no significant association between EBV seropositivity and other potential risk factors for development of allergy (male sex, heredity of allergic diseases, short duration of breastfeeding and low socioeconomic status). Moreover there was no relationship between EBV seropositivity and development of allergic diseases (asthma and suspected allergic rhinitis), sensitization to airborne allergens and food allergens or occurrence of respiratory tract infections. The level of participation in the serologic analysis was 68.4%, and a greater number of the participants had bronchitis than nonparticipants. If nonparticipation was differentially related to EBV infection status between allergic and nonallergic children, or between levels of risk indicators for EBV prevalence, this would have biased the results. However, the assessment of the relation between different risk factors and EBV seroprevalence also included

TABLE 3. Odds ratios and 95% confidence interval for seropositivity to EBV at about age 4 years in relation to risk factors for allergy in the 2561 children from the prospective BAMSE birth cohort with serology available

Risk Factors	No. of Subjects (N = 2561)	Seropositive Children	Crude		Adjusted*	
			OR	95% CI	OR	95% CI
Maternal smoking						
Never	2080	1071 (51.5)†	1.0			
During pregnancy only‡	55	25 (45.5)	0.78	0.46–1.34	0.76	0.45–1.31
At any time after birth of child	374	220 (58.8)	1.35	1.08–1.68	1.29	1.02–1.63
Unknown§	52	31 (59.6)				
Maternal age						
>25 yr of age	2236	1154 (51.6)	1.0			
≤25 yr of age	316	189 (59.8)	1.39	1.10–1.77	1.34	1.04–1.71
Unknown§	9	4 (44.4)				
Gender						
Female	1258	644 (51.2)	1.0			
Male	1303	703 (53.9)	1.12	0.96–1.30	1.12	0.96–1.31
Allergic heredity						
None	1761	933 (52.9)	1.0			
One parent	693	361 (52.1)	0.96	0.81–1.15	0.96	0.80–1.14
Two parents	75	33 (44.0)	0.69	0.44–1.11	0.72	0.45–1.15
Unknown§	32	20 (62.5)				
Breast-feeding						
Un-breast-fed children	57	30 (52.6)	1.0			
<4 mo	159	85 (53.5)	1.03	0.56–1.89	1.03	0.56–1.91
≥4 mo	2305	1210 (52.5)	0.99	0.59–1.68	1.11	0.65–1.90
Unknown§	40	22 (55.0)				
Educational level of the parents						
High¶	1347	699 (51.9)	1.0			
Middle	699	355 (50.8)	0.96	0.79–1.15	0.91	0.76–1.10
Low**	503	287 (57.1)	1.23	1.01–1.51	1.10	0.88–1.37
Unknown§	12	6 (50.0)				

* Adjusted for all other variables in table.

† Numbers in parentheses, percent.

‡ Mothers never smoked after birth of child.

§ Subjects answered questionnaires but no answer was given to this particular item.

¶ One or both parents had held a college or university degree.

|| One or both parents had up to 3 years of upper secondary education (qualifying for college or university studies).

** One or both parents had passed compulsory school (equivalent to primary and lower secondary education) or 2 years of upper secondary education.

TABLE 4. Odds ratios and 95% confidence intervals for outcomes of allergy, sensitization and respiratory tract infections in relation to EBV seropositivity in the 2561 children from the prospective BAMSE birth cohort with serology available, at about 4 years of age

Outcomes	Affected/Not Affected		Crude		Adjusted*	
	EBV-positive Children	EBV-negative Children	OR	95% CI	OR	95% CI
Allergic disease						
Asthma	106/1230	84/1122	1.15	0.85–1.55	1.10	0.81–1.49
Suspected allergic rhinitis	157/1175	139/1055	1.01	0.79–1.29	0.97	0.76–1.25
Respiratory tract infections						
Pneumonia	19/1287	26/1159	0.66	0.36–1.19	0.57	0.30–1.07
Bronchitis	113/1184	91/1085	1.14	0.85–1.52	1.10	0.82–1.47
RSV infection	55/1269	52/1144	0.95	0.65–1.40	0.93	0.63–1.38
Specific IgE antibodies to common allergens						
Phadiatop	192/1155	189/1025	0.90	0.72–1.12	0.90	0.71–1.10
fx5	190/1157	101/1023	0.88	0.71–1.09	0.84	0.69–1.08

* Adjusted for gender, heredity, maternal age, maternal smoking, breast-feeding and parental educational level.

adjustment for potential confounding of the results by cofactors for allergy and EBV.

Previous epidemiologic and serologic surveys^{15–17} have not shown consistent results regarding the role of EBV infection in the development of allergy. Rystedt et al.¹⁶ found that adult patients with atopic dermatitis had elevated titers of antibodies against EBV VCA, compared with controls. Likewise increased titers of EBV antibodies in children 5 to 18 years old with asthma, atopic eczema and rhinoconjunctivitis, compared with nonatopics, have been reported.¹⁵ EBV titers can reflect basic immunoregulatory disturbances. Human B cells, once transformed by EBV, produce and use interleukin-5 in an autocrine manner to induce chronic eosinophilic inflammation and also produce interleukin-4, which has an important role in promoting the production of the IgE antibodies. Thus EBV infection could theoretically lead to production of some major interleukins described in allergy.^{35–37} However, in the allergic child B cells harboring EBV and expressing EBV antigens could be activated, increasing the antigen load and antibody response. It is uncertain whether the elevated EBV titers are a sign that EBV causes the allergy or the result of the allergy.

An Italian study reported an overall higher prevalence of atopics among EBV-seronegative children. Subdivision into age categories indicated that the negative association between atopy and EBV infection was found in the age groups from birth to 6 years, whereas in the 7- to 19-year-old group there was a positive association.¹⁸ In our study a negative association in the younger age group was not reproduced. There might be a need for a long-standing, chronic EBV infection or infection at an older age to trigger allergy. Therefore a positive association between EBV and allergy in older children, as described both by Strannegård¹⁵ and Calvani et al.,¹⁸ is not necessarily contradicted by the present results. Not all allergic diseases or allergen-specific allergies have developed at 4 years of age; further studies in older children could be of value.

Maternal smoking and young maternal age were associated with EBV seropositivity in the present study. EBV seropositivity was more prevalent in children to smoking mothers than in children whose mothers did not smoke. Reasons for this could be either that smoking mothers are more frequently infected with EBV or that they shed more EBV in saliva, thereby becoming more contagious for their children. Because most adults are already EBV-seropositive, the first explanation is less likely. EBV replicates in the oropharyngeal epithelial cells during their terminal stages of differentiation,^{38, 39} and it has been reported that the mucosa of smokers shows a “higher” degree of terminal differentiation than that of nonsmokers.⁴⁰ EBV shedding in saliva in HIV-infected persons has also been reported to be more frequent in tobacco smokers.⁴¹ The association found in this study between EBV infection of children and maternal smoking indicates that it could promote EBV spread by increasing viral shedding. In addition in the association between EBV seroprevalence and smoking, there is a potential that smoking could confound the association between EBV and allergy.

The EBV seroprevalence was higher in children whose mothers were 25 years of age and younger. Increased shedding with more recent primary infection could be an explanation. This also makes mother's age a potential confounder that should be generally accounted for in analyses of EBV and allergy, given that there is evidence suggesting that younger mothers are more likely to have children who develop wheezing illnesses in early life.⁴²

In our large study there was no evidence supporting the hypothesis that EBV infection in early childhood could play an important role in the pathogenesis of allergic diseases or allergen-specific IgE in children at 4 years of age. The association between EBV and both young maternal age and maternal smoking, factors possibly related to allergy, indicates that confounding may bias unadjusted studies.

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