

Cytomegalovirus infection and development of allergic diseases in early childhood: Interaction with EBV infection?

Anna Sidorchuk, MD,^{a,c} Magnus Wickman, MD, PhD,^{a,d} Göran Pershagen, MD, PhD,^{a,d} Frédéric Lagarde, PhD,^a and Annika Linde, MD, PhD^b Stockholm, Sweden, and St Petersburg, Russia

Background: Chronic replication of cytomegalovirus and EBV in early life may affect the immune system and play a role in the development of allergy in children.

Objective: To assess the relation between cytomegalovirus infection and allergic disorders in children, including a possible interaction with EBV infection.

Methods: From a prospective birth cohort study in Stockholm, on factors of importance for development of allergy, 2581 four-year-old children were enrolled. The classification of allergic diseases was based on questionnaire answers and determination of IgE antibodies to common airborne and food allergens. IgG to cytomegalovirus was determined by a commercial ELISA and to EBV by indirect immunofluorescence.

Results: A total of 1191 (46%) children were cytomegalovirus-seropositive. There were no significant associations between seropositivity to cytomegalovirus and allergic manifestations, such as bronchial asthma, suspected allergic rhinitis, or atopic dermatitis. Seropositivity to cytomegalovirus alone, ie, without seropositivity to EBV, was related to IgE antibodies to airborne and food allergens (adjusted odds ratio, 1.8; 95% CI, 1.2–2.9). An antagonism between cytomegalovirus and EBV in relation to sensitization to airborne and food allergens was suggested ($P = .05$).

Conclusion: The study does not support the hypothesis that previous cytomegalovirus infection plays an important role in the pathogenesis of bronchial asthma, suspected allergic rhinitis, or atopic dermatitis in children. However, in the absence of EBV infection, cytomegalovirus infection may be related to sensitization to airborne and food allergens. (J Allergy Clin Immunol 2004;114:1434–40.)

Key words: Asthma, allergy, children, cytomegalovirus, Epstein-Barr virus, risk factor, sensitization, seroprevalence

From ^athe Department of Environmental Epidemiology, Institute of Environmental Medicine, and ^bthe Department of Virology, Swedish Institute for Infectious Diseases Control and Microbiology and Tumorbiology Center, Karolinska Institute, Stockholm; ^cthe Department of Epidemiology, Mechnikov State Medical Academy, St Petersburg; and ^dthe Department of Occupational and Environmental Health, Stockholm County Council, Stockholm.

Supported by grants from the Swedish Institute (New Visby Program), the Swedish Foundation for Health Care Sciences and Allergy Research, the Heart-Lung Foundation, and the Asthma and Allergy Foundation.

Received for publication April 15, 2004; revised July 26, 2004; accepted for publication August 2, 2004.

Available online October 13, 2004.

Reprint requests: Annika Linde, MD, PhD, Department of Virology, Swedish Institute for Infectious Diseases Control and Microbiology and Tumorbiology Center, Karolinska Institute, Solna SE-171 82, Sweden. E-mail: annika.linde@smi.ki.se.

0091-6749/\$30.00

© 2004 American Academy of Allergy, Asthma and Immunology
doi:10.1016/j.jaci.2004.08.009

Abbreviations used

BAMSE: Children (Barn), Allergy, Environment (Miljö), Stockholm, Epidemiological Survey

OR: Odds ratio

OR_{adj}: Adjusted odds ratio

RSV: Respiratory syncytial virus

The prevalence of allergic and hypersensitivity reactions has increased in industrialized parts of the world, particularly among children, for largely unknown reasons.^{1,2} A decline in certain childhood infections and a more general lack of exposure to a broad range of infectious agents in the first years of life have been suggested as contributing factors.^{3,4} A changed spectrum of infections could cause an imbalance between T_H1-mediated and T_H2-mediated immune responses,⁴ contributing to allergy.⁵ There is probably a complicated interplay between infections and the immune system.^{6,7} Not just 1 infecting micro-organism but changes in the pattern of interactions among many agents could affect the development of allergy.

In recent years, much interest has been focused on respiratory syncytial virus (RSV) infections and the development of bronchial asthma.^{8,9} Other viruses such as herpesviruses may also be of importance, but their possible effect has been studied to a limited extent.^{10–12} They are ubiquitous and are chronically excreted or reactivated with varying frequency, possibly affecting the immune system.^{13–15} Cytomegalovirus is a herpesvirus, which seems to favor a T_H1 immune response. Approximately 70% of Swedish mothers of child-bearing age are seropositive to cytomegalovirus.¹⁶ Cytomegalovirus is often transmitted to the child during delivery by infected maternal genital secretion, or afterwards via breast milk.¹⁷ Approximately 1% of children attain new cytomegalovirus infections yearly after 1 year of age.¹⁸ There are few studies addressing the relation between cytomegalovirus infection and subsequent development of allergic disorders,^{11,12,19–22} and the results are inconclusive.

EBV is also ubiquitous and seems to favor a T_H2 immune response by induction of IL-10 in infected cells, production of an IL-10 analogue, and induction of B-cell proliferation as well as polyclonal antibody production.²³ It may thus counteract the immunologic effect of cytomegalovirus. The reports on a role of EBV in atopy are still

inconsistent,^{10,24,25} but it has been shown that EBV infection may lead to production of some major ILs described in allergy,²⁶ and infections have been suggested to result in atopic disease.¹³ Cytomegalovirus and EBV both infect in early childhood in nonindustrialized countries where allergy is uncommon, but are often delayed in industrialized countries.

To elucidate the role of cytomegalovirus infection for development of allergy, the seroprevalence to cytomegalovirus was examined in Swedish 4-year-old children who participated in a large prospective birth cohort survey (BAMSE: Children [Barn], Allergy, Environment [Miljö], Stockholm, Epidemiological Survey).²⁷ Data obtained in a previous study²⁴ on EBV seroprevalence and allergic disorders in the same cohort were also used to assess interaction between EBV and cytomegalovirus infections in relation to allergic disease and sensitization.

METHODS

Study subjects

The study was based on a birth cohort including 4089 infants (2065 boys and 2024 girls) born from February 11, 1994, until November 22, 1996, in Stockholm. The aim was to assess the role of various environmental and lifestyle factors during early childhood for development of allergic disorders. The BAMSE study design has been presented in detail previously.²⁷ Briefly, the parents of the included families answered 4 questionnaires over time concerning risk factors, symptoms, and health outcomes during the first 4 years of life of their children. The questionnaires were answered when the children were approximately 2 months and 1, 2, and 4 years old. The response rate at 4 years was 91.5%.²⁴

The children were also invited to a clinical examination and collection of blood samples at 4 years of age. The median age at participation was 4.3 years. For various reasons, 1128 children were not sampled.²⁴ Among these, 86 had moved out of Stockholm, and 291 families did not answer the invitation. Another 401 families refused blood sampling. Furthermore, it was not possible to draw blood from 350 children who took part in the clinical examination, because only 1 attempt was allowed. Thirty-three samples were excluded from the serologic analyses because of insufficient volume. Eventually, the cytomegalovirus serostatus was determined in 2581 children. The EBV serostatus had previously been determined in the same group of children, but 20 samples were excluded from the final analysis because the results were indeterminate.²⁴ Thus, 2561 children composed the final study group.

Asthma was defined as at least 4 episodes of wheezing without an ongoing cold during the preceding 12 months or at least 1 episode during the same period together with treatment of inhaled corticosteroids.²⁸ Suspected allergic rhinitis was defined as a runny, itchy, or blocked nose without an ongoing cold during the preceding 12 months.²⁹ Atopic dermatitis was defined as an itchy rash with typical distribution ongoing for at least 2 weeks, dry skin, and/or a doctor's diagnosis of eczema.³⁰

Serologic methods

The commercial ELISA Enzygnost Anti CMV/IgG (Dade Behring, Marburg GmbH, Germany) was used for determination of cytomegalovirus IgG antibodies.³¹ The analysis was performed according to the instructions from the manufacturer. The Anti-CMV Reference positive and negative samples provided by the manufacturer were used as reference samples. All references and test

samples were prediluted 1:20 with sample buffer. The quantitative evaluation was performed by the α -method, and an optical density of 0.25 was chosen as a cutoff for positive results, both according to the manufacturer. Repeated analyses to confirm the validity of the Enzygnost Anti CMV/IgG results were performed for examinations showing unusually similar results for all sera included in 1 run. There was no interassay variation concerning serostatus.

IgG antibodies to the EBV capsid antigen remain lifelong after the primary infection, and for determination of the EBV seroprevalence, these antibodies were investigated. An indirect immunofluorescence assay was used according to previous publications.^{24,32}

IgE antibodies to common airborne allergens were determined with Phadiatop (a multiallergen test containing allergens of *Der-matophagoides pteronyssinus* and *Cladosporium*, cat, dog, horse, timothy, birch, mugwort) and to common food allergens with fx5 (containing allergens of milk, fish, eggs, wheat, peanut, and soybean; Pharamacia CAP System; Pharamacia Diagnostics AB, Uppsala, Sweden) according to instructions from the manufacturer. A positive result was defined as an IgE antibody level ≥ 0.35 kilo units per liter.

Statistical methods

Odds ratio (OR) estimates, and corresponding 95% CIs for asthma, atopic dermatitis, suspected allergic rhinitis, signs of sensitization (specific IgE antibodies to common allergens), and respiratory tract infections (pneumonia, bronchitis, and RSV infection) were obtained by using logistic regression and were adjusted for potential risk factors for allergic diseases. These included parental allergy (no parent/1/both), maternal smoking (never/smoking during pregnancy only/at any time after birth of child), maternal age at the birth of the child (mothers at 25 years and younger/older than 25 years), breast-feeding (children who had never been breast-fed/breast-fed less than 4 months/breast-fed 4 months and more), parental educational level (1 or both parents held a college or university degree/1 or both parents had as much as 3 years of upper secondary education, qualifying for college or university studies/lower education). Multinomial logistic regression was used for assessment of outcomes with multiple categories. A likelihood ratio test was performed for estimation of interaction between cytomegalovirus and EBV with respect to specific IgE antibodies to common airborne and food allergens. The statistical package STATA 7.0 was used for analyses.³³

When computing the ORs, the effect of seropositivity to cytomegalovirus was assessed by using all children who were seronegative to cytomegalovirus as a comparison group, whereas for the effect of dual seropositivity to cytomegalovirus and EBV, and the effect of single seropositivity to cytomegalovirus or to EBV, children who were seronegative to both cytomegalovirus and EBV were used as a reference group (Fig 1).

RESULTS

If the prevalence of allergic outcomes and risk factors for development of allergy were differently associated with viral seropositivity in participants and nonparticipants (ie, with and without blood samples, respectively), the study might be biased. To assess such potential bias, the distribution of socioeconomic characteristics, allergic outcomes, and respiratory symptoms among the 2581 participants and in 1161 nonparticipants was studied.²⁴ The only statistically significant differences between the groups were a higher prevalence of bronchitis among the participants (7.9%) than among the nonparticipants

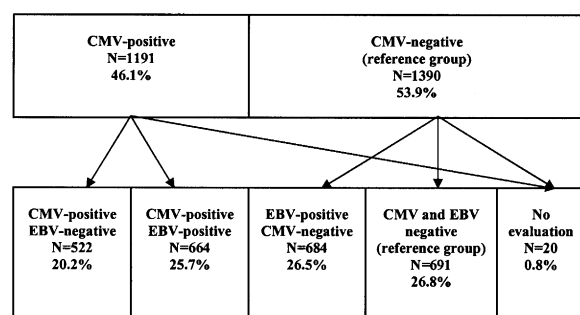


FIG 1. Number of children analyzed for cytomegalovirus and EBV serostatus in the prospective BAMSE birth cohort.

(5.9%) and a higher prevalence of breast-feeding among participants (breast-feeding ≥ 4 months 90.1% vs 87.6% among nonparticipants).

The total number of cytomegalovirus seropositives was 1191 (46.1%; Fig 1). The relation between seropositivity to cytomegalovirus and different risk factors for the development of allergy is presented in Table I. Children whose mothers were ≤ 25 years old were cytomegalovirus-seropositive more frequently than children with older mothers. In the group of children who were breast-fed for 4 months and longer, cytomegalovirus seropositivity was more prevalent compared with children who were breast-fed for a shorter time or not at all. This association was not markedly affected by adjustment for maternal age and maternal educational level. Nineteen of the 57 children (33.3%) who were not breast-fed at all had a positive cytomegalovirus serostatus. Spacious living was negatively associated with cytomegalovirus seropositivity. In children who lived in apartments or houses in which the living area per person was 35 m² and greater, the seroprevalence to cytomegalovirus was lower than in children who lived in apartments with <15 m² per person. There was no association between seroprevalence to cytomegalovirus and number of children at the nursery school or age of entry to day care (data not shown).

No clear association was found between the seroprevalence to cytomegalovirus and asthma, symptoms of suspected allergic rhinitis, or atopic dermatitis (Table II). Neither was there any statistically significant association between seropositivity to cytomegalovirus and occurrence of respiratory infections (pneumonia and bronchitis) or with RSV infection. However, sensitization to common airborne or food allergens tended to be more prevalent in the cytomegalovirus-seropositive group of children, but the association was not significant.

Twenty percent of children were exclusively positive to cytomegalovirus, whereas seropositivity to EBV alone was demonstrated in 26.5% and seropositivity to both cytomegalovirus and EBV in the same individual in 25.7% (Fig 1). Seropositivity to either cytomegalovirus alone or EBV alone as well as seropositivity to both viruses were positively associated with young maternal age (adjusted odds ratio [OR_{adj}], 1.7; 95% CI, 1.2-2.5; OR_{adj}, 1.7; 95% CI, 1.2-2.1; OR_{adj}, 1.9; 95% CI, 1.3-2.8), respectively. Joint seropositivity tended to be inversely

associated to the size of living area per person (35 m² or greater) in the household (OR_{adj}, 0.4; 95% CI, 0.2-0.9; data not shown).

There was no association between outcome of any allergic disease and single seropositivity to either cytomegalovirus or EBV or to joint viral seropositivity (Table III). However, among children seronegative for EBV, children who were seropositive for cytomegalovirus had an increased prevalence of specific IgE to common airborne allergens and an increased joint prevalence of IgE to airborne and food allergens compared with other groups. There was a borderline antagonism between cytomegalovirus and EBV infection in relation to sensitization ($P = .05$).

Additional analyses were performed to assess seropositivity to cytomegalovirus and/or to EBV in relation to asthma, suspected allergic rhinitis, and atopic dermatitis combined with sensitization to airborne or food allergens. Overall, no clear associations were observed. However, there was an association between cytomegalovirus seropositivity and atopic dermatitis in combination with sensitization to food allergens among EBV-negative children (OR_{adj}, 1.9; 95% CI, 1.2-3.2; data not shown).

DISCUSSION

The prevalence of cytomegalovirus seropositivity of approximately 46% in young children found in this study is in line with previous epidemiologic studies in developed countries.^{34,35} The seroprevalence to cytomegalovirus was higher in children whose mothers were 25 years and younger. A possible explanation is that increase of cytomegalovirus shedding including virus in breast milk can be related to more recent primary infections. The increase in primary infection of cytomegalovirus in teenagers and young adults in Western countries, including Sweden, supports this assumption.^{16,36} The finding is also supported by Chandler et al,³⁷ who found a strong correlation between positive cervical cultures for cytomegalovirus and young age in seropositive pregnant women. Fowler et al³⁸ have suggested that biological, age-related effects may contribute to a productive cytomegalovirus infection in mothers during pregnancy. This makes the age of mothers a potential confounder that needs to be accounted for in analyses of cytomegalovirus and allergy, because there is evidence suggesting that younger mothers are more likely to have children who develop wheezing illnesses in early life.³⁹

In our study, cytomegalovirus infection was more prevalent in children who were breast-fed for 4 months and longer compared with other children. Prolonged excretion of virus in breast milk and in other body fluids is characteristic after cytomegalovirus infection and plays an important role in transmission.⁴⁰ Some epidemiologic studies have recently shown that the transmission rate of cytomegalovirus infection from seropositive mothers to preterm infants by breast-feeding is 14% to 44%.¹⁷ Apart from the aforementioned associations, negative relations

TABLE I. ORs and 95% CIs for seropositivity to cytomegalovirus in relation to risk factors for allergy in the 2581 four-year-old children from the prospective BAMSE birth cohort

Risk factors	No. subjects (N = 2581)	Seropositive children (%)	Adjusted*	
			OR	95% CI
Maternal age				
>25 y	2252	1019 (45.3)	1.0	
≤25 y	320	168 (52.5)	1.3	1.1-1.7
Unknown	9	4 (44.4)		
Breast-feeding				
None	57	19 (33.3)	1.0	
<4 months	159	65 (40.9)	1.4	0.7-2.1
≥4 months	2325	1085 (46.7)	1.8	1.0-3.2
Unknown	40	22 (55.0)		
Crowdedness (m ² /person)				
<15	70	40 (57.1)	1.0	
≥15 to <35	2192	1028 (46.9)	0.7	0.4-1.1
≥35 and more	310	119 (38.4)	0.5	0.3-0.8
Unknown	9	4 (44.4)		
Maternal smoking				
Never	2097	970 (46.3)	1.0	
During pregnancy only†	55	28 (50.9)	1.2	0.7-2.0
Ever after birth of child	377	161 (42.7)	0.8	0.7-1.1
Unknown	52	32 (61.5)		
Sex				
Female	1273	592 (46.5)	1.0	
Male	1308	599 (45.8)	0.9	0.8-1.1
Parental allergy				
None	1769	821 (46.4)	1.0	
1 parent	702	317 (45.2)	0.9	0.8-1.1
2 parents	77	37 (48.1)	1.1	0.7-1.7
Unknown	33	16 (48.5)		
Educational level of the parents				
High‡	1357	599 (44.1)	1.0	
Middle§	701	347 (49.5)	1.2	1.0-1.5
Low	511	241 (47.2)	1.1	0.9-1.4
Unknown	12	4 (33.3)		
Gestational age				
≥36 wk	2489	1156 (46.4)	1.0	
<36 wk	83	31 (37.4)	0.8	0.5-1.2
Unknown	9	4 (44.4)		
Number of permanent living brothers/sisters				
0	606	297 (49.0)	1.0	
1	1554	720 (46.3)	0.9	0.8-1.1
2	338	147 (43.5)	0.8	0.6-1.1
3	48	19 (39.6)	0.7	0.4-1.3
4	11	3 (27.3)	0.4	0.1-1.6
5	1	0		
Unknown	23	5 (21.7)		
Attendance at nursery school				
No	162	78 (48.2)	1.0	
Yes	2402	1109 (46.2)	0.9	0.7-1.4
Unknown	18	4 (22.2)		

*Adjusted for other risk factors (maternal age, breast-feeding, sex, parental allergy, maternal smoking, and parental educational level).

†The mothers never smoked after birth of the child.

‡One or both parents held a college or university degree.

§One or both parents had as much as 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.

with the size of living area in apartments or houses per person in the household were observed for children with cytomegalovirus-positive serostatus as well as for doubly infected children. The lack of close or prolonged contact

with playmates who are excreting virus because of non-crowded living conditions might be an explanation.⁴⁰

We did not find convincing evidence that cytomegalovirus infection before the age of 4 years facilitates the

TABLE II. ORs and 95% CIs for outcomes of allergy, signs of sensitization, and respiratory tract infections in relation to cytomegalovirus seropositivity in the 2581 four-year-old children from the prospective BAMSE birth cohort

Outcomes	Cytomegalovirus-negative children		Cytomegalovirus-positive children		
	No. subjects	Affected (%)	No. subjects	Affected (%)	Adjusted* OR (CI 95%)
Allergic diseases					
Asthma	1376	108 (7.8)	1186	85 (7.1)	0.9 (0.7-1.2)
Unknown	14		5		
Suspected allergic rhinitis	1368	152 (10.9)	1276	146 (12.3)	1.1 (0.9-1.5)
Unknown	22		15		
Atopic dermatitis	1389	291 (20.9)	1184	258 (21.7)	1.0 (0.9-1.3)
Unknown	1		7		
Respiratory tract infections					
Pneumonia	1359	23 (1.7)	1152	23 (1.9)	1.1 (0.6-1.9)
Unknown	31		39		
Bronchitis	1352	110 (7.9)	1140	96 (8.1)	1.1 (0.8-1.5)
Unknown	38		51		
RSV infection	1371	64 (4.6)	1169	43 (3.6)	0.8 (0.5-1.2)
Unknown	19		22		
Specific IgE antibodies to common allergens					
Phadiatop	1390	193 (13.9)	1191	197 (16.5)	1.2 (0.9-1.5)
fx5	1390	194 (14.0)	1191	194 (16.3)	1.2 (0.9-1.5)
Both allergens mix tests (Phadiatop and fx5)	1390	87 (6.3)	1191	93 (7.8)	1.3 (0.9-1.7)

*Adjusted for maternal age, breast-feeding, sex, parental allergy, maternal smoking, and parental educational level.

Unknown indicates that the questionnaires were responded to but no answer was given to this particular item.

TABLE III. Prevalence and risk (ORs and 95% CIs) of atopic diseases and allergic sensitization in relation to single and joint seropositivity to cytomegalovirus and EBV in the children from the prospective BAMSE birth cohort at 4 years old

Outcomes	Children negative to both cytomegalovirus and EBV N = 691 (reference group)	Cytomegalovirus-positive, EBV-negative children N = 522		Cytomegalovirus-negative, EBV-positive children N = 684		Cytomegalovirus-positive and EBV-positive children N = 664	
	Affected (%) / not affected	Affected (%) / not affected	Adjusted* OR (CI 95%)	Affected (%) / not affected	Adjusted* OR (CI 95%)	Affected (%) / not affected	Adjusted* OR (CI 95%)
Allergic diseases							
Asthma	50 (7.2)/634	34 (6.5)/486	0.8 (0.5-1.3)	56 (8.2)/621	1.0 (0.7-1.5)	50 (7.5)/611	0.9 (0.6-1.4)
Unknown	7	2		7		3	
Suspected allergic rhinitis	74 (10.7)/606	64 (12.3)/448	1.1 (0.8-1.6)	76 (11.1)/599	0.9 (0.7-1.3)	82 (12.4)/577	1.1 (0.8-1.6)
Unknown	0	2		1		5	
Atopic dermatitis	138 (20.0)/553	123 (23.6)/397	1.2 (0.9-1.6)	148 (21.6)/535	1.1 (0.8-1.4)	133 (20.0)/526	1.0 (0.8-1.3)
Unknown	0	2		1		5	
Specific IgE antibodies to common allergens							
Phadiatop	91 (13.2)/600	96 (18.4)/426	1.4 (1.0-1.9)	91 (13.9)/589	1.0 (0.7-1.4)	99 (14.9)/565	1.1 (0.8-1.5)
fx5	95 (13.8)/596	95 (18.0)/428	1.3 (0.9-1.8)	95 (13.6)/591	0.9 (0.7-1.3)	99 (14.9)/565	1.1 (0.8-1.5)
Both allergens mix tests (Phadiatop and fx5)	37 (5.4)/654	51 (9.8)/471	1.8 (1.2-2.9)	44 (6.4)/640	1.1 (0.7-1.8)	41 (6.2)/623	1.1 (0.7-1.8)

*Adjusted for maternal age, breast-feeding, sex, parental allergy, maternal smoking, and parental educational level.

development of asthma or rhinitis. In the literature, varying results concerning the relation between allergy and cytomegalovirus infections have been presented. Cytomegalovirus has been reported to be associated with asthma exacerbation in adults.²⁰ A case of cow's milk allergy associated with cytomegalovirus infection in an immunocompetent infant has also been reported, but there was no evidence that cytomegalovirus facilitated the

allergic reaction.¹⁹ Two recent studies indicate that reactivation of cytomegalovirus may contribute to the development of hypersensitivity.^{21,22}

EBV, like cytomegalovirus, causes a chronic infection in children, and these viruses may interact. Our study supports a potential role of early cytomegalovirus infection in sensitization, both to inhalant and food allergens and in atopic dermatitis with sensitization, especially to

food allergens, in cytomegalovirus-positive/EBV-negative children. One recent study supports a relation between cytomegalovirus and atopic dermatitis or sensitization to common allergens.¹² Some epidemiologic studies have shown that exclusive breast-feeding for longer than 4 months may delay the onset or protect against development of respiratory allergy in early life, but the relation between breast-feeding and the development of atopic dermatitis is still controversial and varies in different age groups and different countries.⁴¹ Isolauri et al⁴² showed that breast-feeding may maintain allergic symptoms in sensitized infants. A role of cytomegalovirus infection via breast-feeding in this context deserves further attention.

A greater degree of expansion of CD45RO memory T-cell production has been shown in atopic than in nonatopic children.⁶ It has also been described that CD45RO is usually expressed in response to cytomegalovirus antigens.⁴³ The memory cells produce 10-fold more IFN- γ compared with naive T cells⁴⁴ and may divert T_H1/T_H2 balance to the T_H1 direction. An early cytomegalovirus infection could thus be of importance for the development of atopy.⁶ However, in recent studies, a difference in neither the T_H1 response nor seroprevalence to cytomegalovirus between atopic and nonatopic children was noted.^{11,12} However, these studies do not consider viral interactions.

The associations between seropositivity and the signs of sensitization were found only in children seropositive to cytomegalovirus who were not seropositive to EBV. These 2 viruses may theoretically be counteracting the effect of each other on the immune system by their different T_H1 and T_H2 activations.⁴⁵ Recent studies have shown that the human immune response to cytomegalovirus includes positive staining of T cells for IL-2, TNF- α , IFN- γ , and small amounts of IL-4,⁴⁶ suggesting that cytomegalovirus elicits a prototype T_H1 response. EBV infection, in contrast, may transform human B cells, resulting in production IL-5, which in turn may induce a chronic eosinophilic inflammation, promoting allergy.⁴⁷ Because T_H1-type cytokines inhibit the production of T_H2-type cytokines and vice versa,⁵ the immunologic aspects of coinfection with the 2 viruses might be of specific interest. Our results indicate an antagonistic interaction between cytomegalovirus and EBV in relation to sensitization. This may partly explain heterogeneous findings in different studies on the effect of breast-feeding and underlines the importance of studies of interaction between viral infections.

The authors thank all colleagues from the Department of Occupational and Environmental Health, Stockholm County Council, Stockholm, who have worked in the BAMSE project. Furthermore, we express our gratitude to Professor Marianne V. Hage-Hamsten from the Department of Clinical Immunology, Karolinska Hospital, Stockholm, for preparing all of the tests of allergic sensitization.

REFERENCES

1. Busse WW. Mechanisms and advances in allergic diseases. *J Allergy Clin Immunol* 2000;105:S593-8.

2. Beasley R, Crane J, Lai CK, Pearce N. Prevalence and etiology of asthma. *J Allergy Clin Immunol* 2000;105:S466-72.
3. Martinez FD. Maturation of immune responses at the beginning of asthma. *J Allergy Clin Immunol* 1999;103:355-61.
4. Message SD, Johnston SL. Viruses in asthma. *Br Med Bull* 2002;61:29-43.
5. Umetsu DT, Akbari O, DeKruyff RH. Regulatory T cells control the development of allergic disease and asthma. *J Allergy Clin Immunol* 2003;112:480-7.
6. Smart JM, Kemp AS. Increased Th1 and Th2 allergen-induced cytokine responses in children with atopic disease. *Clin Exp Allergy* 2002;32:796-802.
7. Sheikh A, Smeeth L, Hubbard R. There is no evidence of an inverse relationship between Th2-mediated atopy and Th1-mediated autoimmune disorders: lack of support for the hygiene hypothesis. *J Allergy Clin Immunol* 2003;111:131-5.
8. Gern JE, Busse WW. The role of viral infections in the natural history of asthma. *J Allergy Clin Immunol* 2000;106:201-12.
9. von Mutius E. The environmental predictors of allergic disease. *J Allergy Clin Immunol* 2000;105:9-19.
10. Calvani M, Alessandri C, Paolone G, Rosengard L, Di Caro A, De Franco D. Correlation between Epstein-Barr virus antibodies, serum IgE and atopic disease. *Pediatr Allergy Immunol* 1997;8:91-6.
11. Laske N, Kern F, Nickel R, Wahn U, Volk HD. No difference in type 1 T-cell immune responses to human cytomegalovirus antigens between atopic children and nonatopic children. *J Allergy Clin Immunol* 2003;112:210-2.
12. Laske N, Volk HD, Liebenhalb C, Gruber C, Sommerfeld C, Nickel R, et al. Infantile natural immunization to herpes group viruses is unrelated to the development of asthma and atopic phenotypes in childhood. *J Allergy Clin Immunol* 2002;110:811-3.
13. Okudaira H, Mori A. Concepts of the pathogenesis of allergic disease: possible roles of Epstein-Barr virus infection and Interleukin-2 production. *Int Arch Allergy Immunol* 1999;120:177-84.
14. Lucht E, Biberfeld P, Linde A. Epstein-Barr virus (EBV) DNA in saliva and EBV serology of HIV-1-infected persons with and without hairy leukoplakia. *J Infect* 1995;31:189-94.
15. Chen SF, Tu WW, Sharp MA, Tongson EC, He XS, Greenberg HB, et al. Antiviral CD8 T cells in the control of primary human cytomegalovirus infection in early childhood. *J Infect Dis* 2004;189:1619-27.
16. Ahlfors K, Ivarsson SA, Harris S. Report on a long-term study of maternal and congenital cytomegalovirus infection in Sweden: review of prospective studies available in the literature. *Scand J Infect Dis* 1999;31:443-57.
17. Numazaki K, Chiba S, Asanuma H. Transmission of cytomegalovirus. *Lancet* 2001;357:1799-800.
18. Demmler GJ. Summary of a workshop on surveillance for congenital cytomegalovirus disease. *Rev Infect Dis* 1991;13:315-29.
19. Jonkhoff-Slok TW, Veenhoven RH, de Graeff-Meeder ER, Buller HA. An immunocompetent infant with cow's milk allergy and cytomegalovirus colitis. *Eur J Pediatr* 1997;156:528-9.
20. Atmar RL, Guy E, Guntupalli KK, Zimmerman JL, Bandi VD, Baxter BD, et al. Respiratory tract viral infections in inner-city asthmatic adults. *Arch Intern Med* 1998;158:2453-9.
21. Aihara M, Sugita Y, Takahashi S, Nagatani T, Arata S, Takeuchi K, et al. Anticonvulsant hypersensitivity syndrome associated with reactivation of cytomegalovirus. *Br J Dermatol* 2001;144:1231-4.
22. Docke WD, Kiessling C, Worm M, Friedrich M, Pruss A, Weitz M, et al. Subclinical activation of latent cytomegalovirus (CMV) infection and anti-CMV immune response in patients with atopic dermatitis. *Br J Dermatol* 2003;148:954-63.
23. Klein SC, Kube D, Abts H, Diehl V, Tresch H. Promotion of IL8, IL10, TNF α and TNF β production by EBV infection. *Leuk Res* 1996;20:633-6.
24. Sidorchuk A, Lagarde F, Pershagen G, Wickman M, Linde A. Epstein-Barr virus infection is not associated with development of allergy in children. *Pediatr Inf Dis J* 2003;22:642-7.
25. Strannegard I-L, Strannegard O. Epstein-Barr virus antibodies in children with atopic disease. *Int Arch Allergy Appl Immun* 1981;64:314-9.
26. Ohnishi E, Iwata T, Inouye S, Kurata T, Sairenji T. Interleukin-4 production in Epstein-Barr virus-transformed B cell lines from peripheral

- mononuclear cells of patients with atopic dermatitis. *J Interferon Cytokine Res* 1997;17:597-602.
27. Almqvist C, Egmar AC, van Hage-Hamsten M, Berglund N, Pershagen G, Nordvall SL, et al. Heredity, pet ownership, and confounding control in a population-based birth cohort. *J Allergy Clin Immunol* 2003;111:800-6.
 28. Bel EH. Clinical phenotypes of asthma. *Curr Opin Pulm Med* 2004;10:44-50.
 29. Asher MI, Keil U, Anderson HR, Beasley R, Crane J, Martinez F, et al. International Study of Asthma and Allergies in Childhood (ISAAC): rationale and methods. *Eur Respir J* 1995;8:483-91.
 30. Böhme M, Lannerö E, Wickman M, Nordvall SL, Wahlgren CF. Atopic dermatitis and concomitant disease pattern in children up to two years of age. *Acta Derm Venerol* 2002;82:98-103.
 31. Gutierrez J, Maroto MD, Piedrola G. Evaluation of a new reagent for anti-cytomegalovirus and anti-Epstein-Barr virus immunoglobulin G. *J Clin Microbiol* 1994;32:2603-5.
 32. Linde A, Andersson J, Lundgren G, Wahren B. Subclass reactivity to Epstein-Barr Virus capsid antigen in primary and reactivated EBV infections. *J Med Virol* 1987;21:109-21.
 33. STATA Statistical Software. Release 7.0. College Station, TX: STATA Corp; 2001.
 34. Aarnisalo J, Ilonen J, Vainionpää R, Volanen I, Kaitosaari T, Simell O. Development of antibodies against cytomegalovirus, varicella-zoster virus and herpes simplex virus in Finland during the first eight years of life: a prospective study. *Scand J Infect Dis* 2003;35:750-3.
 35. Grillner L, Strangert K. A prospective molecular epidemiological study of cytomegalovirus infections in two day care centers in Sweden: no evidence for horizontal transmission within the centers. *J Infect Dis* 1988;157:1080-3.
 36. Andersson-Ellstrom A, Svennerholm B, Forsman L. Prevalence of antibodies to herpes simplex virus types 1 and 2, Epstein-Barr virus and cytomegalovirus in teenage girls. *Scand J Infect Dis* 1995;27:315-8.
 37. Chandler SH, Alexander ER, Holmes KK. Epidemiology of cytomegaloviral infection in a heterogeneous population of pregnant women. *J Infect Dis* 1985;152:249-56.
 38. Fowler KB, Stagno S, Pass RF. Maternal age and congenital Cytomegalovirus infection: screening of two diverse newborn populations, 1980-1990. *J Infect Dis* 1993;168:552-6.
 39. Martinez FD. Maternal risk factors in asthma. *Ciba Found Symp* 1997;206:233-9.
 40. Gershon AA, Gold E, Nankervis GA. Cytomegalovirus. In: Evans AS, Kaslow RA, editors. *Viral infections of humans*. 4th ed. New York: Plenum Publishing; 1997. p. 229-51.
 41. Gdalevich M, Mimouni D, David M, Mimouni M. Breast-feeding and the onset of atopic dermatitis in childhood: a systematic review and meta-analysis of prospective studies. *J Am Acad Dermatol* 2001;45:520-7.
 42. Isolauri E, Tabvanainen A, Peltola T, Arvola T. Breast-feeding of allergic infants. *J Pediatr* 1999;134:27-32.
 43. Vargas AL, Lechner F, Kantzanou M, Phillips RE, Klenerman P. Ex vivo analysis of phenotype and TCR usage in relation to CD45 isoform expression on cytomegalovirus-specific CD8⁺ T lymphocytes. *Clin Exp Immunol* 2001;125:432-9.
 44. Wilson CB. The ontogeny of T lymphocyte maturation and function. *J Pediatr* 1991;118:S4-9.
 45. Prescott SL, Macaubas C, Smallacombe T, Holt BJ, Sly PD, Holt PG. Development of allergen specific T-cell memory in atopic and normal children. *Lancet* 1999;353:196-200.
 46. Kallas EG, Reynolds K, Andrews J, Fitzgerald T, Kasper M, Menegus M, et al. Cytomegalovirus-specific IFN γ and IL-4 are produced by antigen expanded human blood lymphocytes from seropositive volunteers. *Immunol Lett* 1998;64:63-9.
 47. Baumann MA, Paul CC. Interleukin-5 is an autocrine growth factor for Epstein-Barr virus-transformed B-lymphocytes. *Blood* 1992;79:1763-7.

Availability of Journal back issues

As a service to our subscribers, copies of back issues of *The Journal of Allergy and Clinical Immunology* for the preceding 5 years are maintained and are available for purchase until inventory is depleted. Please write to Elsevier Inc, Subscription Customer Service, 6277 Sea Harbor Dr, Orlando, FL 32887, or call (800) 654-2452 or (407) 345-4000 for information on availability of particular issues and prices.