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The influence of Epstein-Barr virus and cytomegalovirus on childhood respiratory health: A population-based prospective cohort study

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Abstract

Background: Epstein-Barr virus (EBV) and cytomegalovirus (CMV) infection are common in early childhood. CMV infection favours a T-helper-1 and EBV infection a T-helper-2 cell response, possibly leading to disbalanced T-helper cell response, and subsequent risk of asthma or atopy.

Objective: To study the associations of EBV and CMV with lung function, asthma and inhalant allergic sensitization at school age.

Methods: This study among 3546 children was embedded in a population-based prospective cohort. At age 6 years, serum IgG levels against EBV and CMV were measured by ELISA. At age 10 years, lung function was measured by spirometry, asthma by questionnaire and inhalant allergic sensitization by skin prick test.

Results: Unadjusted models showed that seropositivity for EBV was associated with a higher FEV_1 and FEF_{75} (*Z*-score difference (95% CI): 0.09 (0.02, 0.16) and 0.09 (0.02, 0.15)), while seropositivity for CMV was not. Specific combinations of viruses showed that seropositivity for EBV was only associated with FEV_1 and FEF_{75} in the presence of seropositivity for CMV (0.12 (0.04, 0.20)) and 0.08 (0.01, 0.15)). Seropositivity for CMV in the absence of seropositivity for EBV was associated with an increased risk of inhalant allergic sensitization (OR (95% CI): 1.31 (1.02, 1.68)). All effect estimates attenuated into non-significant mainly after adjustment for child's ethnicity. Seropositivity for EBV or CMV was not associated with asthma.

data collection and analysis, interpretation of data, writing of this report or the decision to submit the article for publication.

Conclusions and Clinical Relevance: Associations of EBV and CMV infections in early childhood with school-age lung function and inhalant allergic sensitization are explained by ethnicity, or sociodemographic and lifestyle-related factors.

KEYWORDS

asthma, child, epidemiology, herpesviridae, respiratory function test

1 | INTRODUCTION

Infectious diseases in early life are suggested to influence the risk of lower lung function and asthma in later childhood and adulthood.[1-4] Epstein-Barr virus (EBV) and cytomegalovirus (CMV) are herpesviridae that are commonly present. Between 60% and 90% of the adults are seropositive with the peak of infections occurring in childhood, and result in life-long viral persistence.[5-7] Successful viral suppression is dependent on expanded T cell memory population. Both viruses primarily affect immune cells, but do not have the same effects. EBV favours a T-helper-2 cell-mediated response and mostly seems to affect B-cells.[8,9] CMV has a T-helper-1 cell-mediated response, and impacts on T cell and natural killer (NK) cell differentation.[10,11] Moreover, both viruses drive memory T cell expansions in young children.[12,13] Therefore, infections with these viruses could lead to a disbalance in immune responses, specifically T-helper cell-mediated responses, and subsequently an increased risk of asthma.[2-4] A prospective cohort study demonstrated that EBV coinfection enhances immune maturation driven by CVM.[14] Two other studies found that children with EBV had a reduced antibody response to measles or rubella vaccination, while children co-infected with CMV had not.[15,16] This further suggests that these herpesviridae might have interacting effects on the immune system. Results from previous studies on the associations of seropositivity for EBV or CMV with asthma and atopy are inconsistent.[2,4,17] This might be explained by the examination of EBV or CMV solely, while co-infections of these viruses might be more important given their difference in immune response.[3,4] This is supported by a cohort study that demonstrated that CMV in the absence of EBV at the age of 4 years was associated with an increased risk of specific IgE of inhalant allergens, or inhalant and food allergens combined, but not asthma.[3] Studies on associations of EBV or CMV with atopic outcomes at older ages in childhood, including more objective respiratory measures such as lung function, are lacking.

Therefore, we examined the associations of childhood EBV or CMV, solely and in combination, with school-age lung function,

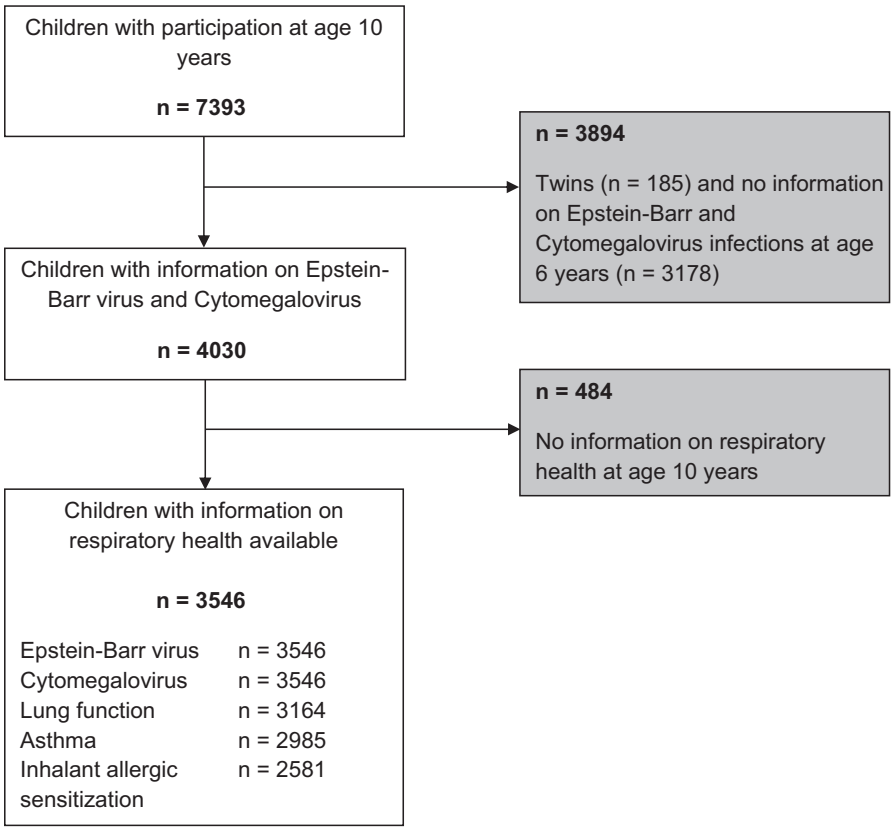

FIGURE 1 Flow chart of participants included for analysis

asthma and inhalant allergic sensitization in a population-based prospective cohort study among 3546 children.

2 | METHODS

2.1 | Design

This study was embedded in the Generation R Study, a population-based prospective cohort from early fetal life onwards in Rotterdam, the Netherlands.[18] The study is designed to identify early environmental and genetic causes and causal pathways leading to normal and abnormal growth, development and health from fetal life to childhood and young adulthood. In total, 9778 mothers with a delivery date from April 2002 until January 2006 were enrolled in the study. Response at baseline was 61%, and general follow-up rates until the age of 10 years were around 80%. The study has been approved by the Medical Ethical Committee of the Erasmus MC, University Medical Center Rotterdam, Rotterdam, The Netherlands (MEC-2012-165). Written informed consent was obtained from parents or legal representatives of all children. Of the 7393 children with participation at age 10 years, we excluded twins (n = 185), children without information on EBV or CMV (n = 3709), and without information on respiratory health (n = 484), which left 3546 children for the current analysis (Figure 1).

2.2 | EBV and CMV

Venous blood samples were obtained by antecubital venipuncture during a visit at the research centre (median age 6.0 years, 5%-95% range 5.7-7.2 years). The response rate for serum samples was 69%. Samples were stored for a maximum of 4 hours at 4°C, and transported twice daily for further processing and storage. Serum samples were analysed using ELISA for IgG antibodies against EBV capsid antigen (native mixture of several viral capsid antigens) and CMV (purified native antigens strain "AD169") (EUROIMMUN). As described earlier, seropositivity was defined as a sample-treshold ratio above 0.8 for EBV and 0.6 for CMV.[12] The presence of seropositivity for the viruses was further combined into neither, EBV only, CMV only, and both EBV and CMV.

2.3 | Respiratory health

We performed spirometry according to the American Thoracic Society (ATS) and European Respiratory Society (ERS) recommendations during a visit at the research centre (median age 9.7 years, 5%-95% range 9.5-10.3 years). Lung function measures included Forced Expiratory Volume in 1 second (FEV_1), Forced Vital Capacity (FVC), FEV_1/FVC and Forced Expiratory Flow after exhaling 75% of FVC (FEF_{75}) and were converted into sex-, height-, age- and ethnicity-adjusted *z*-scores according to the Global Lung Initiative reference data.[19] Children (n = 239) with a >5%, instead of ≤5%, deviation in FEV_1 and FVC, and at least one blow with adequate reach and duration of plateau according to ATS/ERS criteria were also included. The effect estimates for the associations did not differ when we in- or excluded those children.[20,21] Information on asthma medication use in the past 12 months was obtained during the research centre visit. Asthma was defined as ever diagnosis of asthma, obtained by questionnaire at the age 10 years, with either wheezing or asthma medication use in the past 12 months. Questions on wheezing and asthma were based on the International Study on Asthma and Allergy in Childhood (ISAAC) Questionnaire obtained by post ("Has your child suffered from attacks of wheezing in the chest in the past 12 months?" (no/yes, <4 attacks/yes ≥4 attacks) and "Has your child ever had asthma diagnosed by a doctor?" (no/yes <3 years/yes 3-6 years/yes >6 years of age)).[22] Information on medication use was obtained by a short questionnaire during the visit at the research centre ("Did your child receive any prescribed medication in the past 12 months for complaints of the airways, lungs, allergy or skin? If yes, which medication was this?" (no/yes: <name prescribed drug>)). Median time between the questionnaire and visit to the research centre was 1.4 (5%-95% range −6 to 12) weeks. As described earlier, inhalant allergic sensitization at the age of 10 years was measured by skin prick test (SPT), using the scanned area method.[23] Inhalant allergens included house-dust mite, 5-grass mixture, birch, cat and dog (ALK-Albelló BV). Children were considered to have inhalant allergic sensitization when they were sensitized to at least one inhalant allergen.

2.4 | Covariates

At enrolment, information on maternal age, ever history of asthma and atopy (no/yes), educational level (low or middle/high), body mass index, parity and current pet keeping (no/yes) was obtained by questionnaires during pregnancy. Information on maternal smoking during pregnancy was obtained in early, mid and late pregnancy by questionnaires, and combined into no smoking or quitting in early pregnancy/continued smoking. Psychological distress during mid-pregnancy was measured by the Brief Symptom inventory.[24] Child's gestational age at birth and birth weight were obtained from midwife and hospital records at birth. Child's ethnicity was based on country of birth of the parents from parental questionnaires at enrolment, and combined (European/non-European). Questionnaires in the first year of life provided information on ever breastfeeding (no/yes) and daycare attendance in the first year of life (no/yes).

2.5 | Statistical analysis

First, we compared characteristics of mothers and children included in the study to those lost to follow-up. Next, we studied the associations of both EBV and CMV solely and combined (neither, EBV only, CMV only or both EBV and CMV) with lung function,

TABLE 1 Characteristics of children and their mothers

	n = 3546
Maternal characteristics	
Age (y)	31.2 (4.9)
History of asthma or atopy, yes (%)	36.2 (1285)
Educational level, low/middle (%)	50.3 (1751)
Body mass index at intake (kg/m^2)	24.6 (4.2)
Parity, nulliparous (%)	55.5 (1967)
Psychological distress during pregnancy[a]	0.17 (0.00, 0.93)
Smoking during pregnancy, yes (%)	14.4 (512)
Pet keeping, yes (%)	36.4 (1290)
Child's characteristics	
Female sex (%)	48.8 (1729)
Gestational age at birth (wks)[a]	40.1 (37.0, 42.0)
Birth weight (g)	3447 (546)
Ethnicity, European (%)	67.7 (2399)
Ever breastfeeding, yes (%)	91.7 (3252)
Day care attendance 1st y, yes (%)	55.4 (1966)
EBV IgG at 6 y, positive (%)	50.4 (1787)
CMV IgG at 6 y, positive (%)	41.5 (1470)
Combination of infections (%)	
Neither	32.6 (1155)
EBV only	26.0 (921)
CMV only	17.0 (604)
Both	24.4 (866)
Lung function measures	
FEV_1 (L)	2.02 (0.30)
FVC (L)	2.34 (0.36)
FEV_1/FVC (%)	87.0 (5.6)
FEF_{75} (L/s)	1.15 (0.35)
Asthma, yes (%)	5.8 (172)
Inhalant allergic sensitization, yes (%)	31.7 (818)

Note: Values are means (SD), valid percentages (absolute numbers) or [a]medians (5-95th percentiles). Values are based on imputed data. Data was missing and not imputed for lung function measures (n = 382), asthma (n = 561), and inhalant allergic sensitization (n = 965).

Abbreviations: CMV, Cytomegalovirus; EBV, Epstein-Barr virus; FEF_{75}, forced expiratory flow after exhaling 75% of FVC; FEV_1, forced expiratory volume in the first second; FVC, forced vital capacity.

and asthma and inhalant allergic sensitization by using linear and logistic regression models, respectively. All models were adjusted for confounders, which were selected from literature, and were associated with the exposure and the outcome, or changed the effect estimates of univariate analyses with 10% or more when added to the crude model. Confounders were grouped into sociodemographic and health-related factors (maternal age, history of asthma and atopy, educational level, parity and psychological distress during pregnancy), lifestyle-related factors (maternal body mass index, smoking during pregnancy and pet keeping, and child's birth weight adjusted for gestational age, breastfeeding and daycare attendance) and child's ethnicity. Models were adjusted for each group of confounders separately, and finally for all groups of confounders. The percentage of missing data in confounders was between 1.7% and 24.8%, except for daycare attendance (37.9%). Missing data were imputed by multiple imputation using chained equations to select the most likely value for a missing response, creating ten new datasets. Since we observed no major differences in the magnitude or direction of the effect estimates between analyses with imputed missing data and complete cases only, we only present the results based on imputed datasets. All measures of association are presented as odds ratios (OR) or *Z*-score differences and their corresponding 95% confidence intervals (95% CI). Statistical analyses were performed using SPSS version 25.0 for Windows software (IBM Corp).

3 | RESULTS

3.1 | Subject characteristics

Characteristics of children and their mothers are presented in Table 1. At the age of 6 years, 50.4% (n = 1787) of the children were seropositive for EBV, and 41.5% (n = 1470) for CMV. When we combined the viruses, 32.6% (n = 1155) of the children was seropositive for none of the viruses, 26.0% (n = 921) for EBV only, 17.0% (n = 604) for CMV only and 24.4% (n = 866) for both. The prevalence of current asthma at the age of 10 years was 5.8% (n = 172), and of inhalant allergic sensitization 31.7% (n = 818). Children not included in the analysis had mothers who were younger and lower educated, had a higher body mass index and had more psychological distress during pregnancy. Children were more often of non-European ethnicity, had a lower gestational age and birth weight, and were more likely to be seropositive for EBV, but not CMV (Table S1).

3.2 | EBV, CMV and respiratory health

In the unadjusted analyses, EBV, but not CMV, was associated with a higher FEV_1 and FEF_{75} (*Z*-score difference (95% confidence interval): 0.09 (0.02, 0.16) and 0.09 (0.02, 0.15), respectively) (Table 2). Combinations of seropositivity for the viruses showed that only seropositivity for EBV in the presence of seropositivity for CMV was associated with higher FEV_1, FVC and FEF_{75} (*Z*-score difference (95% CI): 0.12 (0.04, 0.20), 0.10 (0.02, 0.17) and 0.08 (0.01, 0.15), respectively)). Seropositivity for EBV or CMV, or combinations of seropositivity for the viruses were not associated with asthma. Seropositivity for EBV or CMV solely were not associated with inhalant allergic sensitization. Combinations of seropositivity for the viruses demonstrated that only seropositivity for CMV in the absence of seropositivity for EBV was associated with an increased risk of inhalant allergic sensitization (OR (95% CI): 1.29 (1.01, 1.65). After adjustment for confounders, all effect estimates attenuated

TABLE 2 Unadjusted associations of EBV and CMV with respiratory health

	FEV_1 Z-score difference (95% Confidence Interval) n = 3164	FVC Z-score difference (95% Confidence Interval) n = 3164	FEV_1/FVC Z-score difference (95% Confidence Interval) n = 3164	FEF_{75} Z-score difference (95% Confidence Interval) n = 3164	Asthma Odds ratio (95% Confidence Interval) n = 2985	Inhalant allergic sensitization Odds ratio (95% Confidence Interval) n = 2581
EBV						
Seronegative	Reference	Reference	Reference	Reference	Reference	Reference
Seropositive	**0.09 (0.02, 0.16)***	0.06 (−0.00, 0.13)	0.06 (−0.01, 0.12)	**0.09 (0.02, 0.15)****	1.13 (0.83, 1.54)	1.05 (0.89, 1.23)
CMV						
Seronegative	Reference	Reference	Reference	Reference	Reference	Reference
Seropositive	0.07 (−0.00, 0.14)	0.06 (−0.00, 0.13)	0.01 (−0.06, 0.08)	0.02 (−0.04, 0.09)	0.96 (0.70, 1.32)	1.11 (0.94, 1.31)
Combination of viruses						
Neither	Reference	Reference	Reference	Reference	Reference	Reference
EBV only	0.01 (−0.07, 0.08)	−0.02 (−0.09, 0.06)	0.04 (−0.04, 0.12)	0.04 (−0.04, 0.11)	1.20 (0.81, 1.78)	1.17 (0.94, 1.65)
CMV only	−0.04 (−0.13, 0.05)	−0.02 (−0.11, 0.07)	−0.02 (−0.11, 0.07)	−0.07 (−0.15, 0.02)	1.02 (0.64, 1.62)	**1.29 (1.01, 1.65)***
Both	**0.12 (0.04, 0.20)****	**0.10 (0.02, 0.17)***	0.04 (−0.04, 0.11)	**0.08 (0.01, 0.15)***	1.07 (0.70, 1.62)	1.12 (0.90, 1.40)

Note: Values are Z-score differences or odds ratios with 95% confidence interval, derived from unadjusted linear or logistic regression models, respectively.

Abbreviations: CMV, Cytomegalovirus; EBV, Epstein-Barr virus; FEF_{75}, forced expiratory flow when 75% of the FVC is exhaled; FEV_1, forced expiratory flow in 1 s; FVC, forced vital capacity.

*P-value < .05.

**P-value < .01.

Bold values represent significant associations.

TABLE 3 Adjusted associations of combinations of EBV and CMV with respiratory health

	FEV_1 *Z*-score difference (95% Confidence Interval) n = 3164	FVC *Z*-score difference (95% Confidence Interval) n = 3164	FEV_1/FVC *Z*-score difference (95% Confidence Interval) n = 3164	FEF_{75} *Z*-score difference (95% Confidence Interval) n = 3164	Asthma Odds ratio (95% Confidence Interval) n = 2985	Inhalant allergic sensitization Odds ratio (95% Confidence Interval) n = 2581
Sociodemographic and health-related factors[a]						
Neither	Reference	Reference	Reference	Reference	Reference	Reference
EBV only	−0.00 (−0.08, 0.07)	−0.02 (−0.10, 0.05)	0.03 (−0.05, 0.11)	0.02 (−0.05, 0.09)	1.10 (0.74, 1.64)	1.14 (0.92, 1.43)
CMV only	−0.03 (−0.12, 0.06)	−0.01 (−0.10, 0.07)	−0.01 (−0.10, 0.07)	−0.05 (−0.13, 0.04)	0.99 (0.62, 1.59)	1.30 (1.00, 1.64)
Both	**0.12 (0.04, 0.20)****	**0.10 (0.03, 0.18)***	0.02 (−0.05, 0.10)	0.06 (−0.01, 0.14)	0.95 (0.61, 1.47)	1.10 (0.88, 1.38)
Lifestyle-related factors[b]						
Neither	Reference	Reference	Reference	Reference	Reference	Reference
EBV only	0.01 (−0.07, 0.09)	−0.01 (−0.09, 0.06)	0.04 (−0.03, 0.12)	0.04 (−0.04, 0.11)	1.13 (0.68, 1.77)	1.12 (0.90, 1.41)
CMV only	−0.04 (−0.13, 0.05)	−0.01 (−0.10, 0.07)	−0.03 (−0.12, 0.06)	−0.07 (−0.15, 0.02)	1.13 (0.76, 1.68)	1.24 (0.97, 1.59)
Both	**0.11 (0.03, 0.19)****	**0.09 (0.01, 0.16)***	0.03 (−0.05, 0.11)	0.07 (−0.00, 0.15)	1.08 (0.70, 1.66)	1.06 (0.85, 1.33)
Ethnicity[c]						
Neither	Reference	Reference	Reference	Reference	Reference	Reference
EBV only	0.01 (−0.07, 0.09)	−0.01 (−0.08, 0.06)	0.04 (−0.03, 0.12)	0.04 (−0.03, 0.11)	1.10 (0.74, 1.63)	1.11 (0.89, 1.39)
CMV only	−0.04 (−0.13, 0.04)	−0.02 (−0.11, 0.06)	−0.03 (−0.07, 0.02)	0.11 (−0.15, 0.01)	0.94 (0.59, 1.48)	1.23 (0.96, 1.56)
Both	0.02 (−0.06, 0.10)	0.01 (−0.07, 0.08)	0.01 (−0.07, 0.09)	−0.01 (−0.07, 0.06)	0.89 (0.58, 1.37)	0.99 (0.79, 1.25)
Fully adjusted model[d]						
Neither	Reference	Reference	Reference	Reference	Reference	Reference
EBV only	0.02 (−0.06, 0.09)	−0.01 (−0.08, 0.07)	0.04 (−0.04, 0.12)	0.04 (−0.03, 0.12)	1.04 (0.69, 1.56)	1.09 (0.87, 1.37)
CMV only	−0.05 (−0.14, 0.04)	−0.03 (−0.11, 0.06)	−0.03 (−0.12, 0.06)	−0.07 (−0.16, 0.01)	0.99 (0.61, 1.60)	1.18 (0.92, 1.52)
Both	0.03 (−0.05, 0.11)	0.03 (−0.05, 0.10)	0.00 (−0.08, 0.08)	−0.01 (−0.09, 0.06)	0.90 (0.57, 1.41)	0.97 (0.77, 1.23)

Note: Values are *Z*-score differences or odds ratios with 95% confidence interval, derived from linear or logistic regression models, respectively.

Abbreviations: CMV, Cytomegalovirus; EBV, Epstein-Barr virus; FEF_{75}, forced expiratory flow when 75% of the FVC is exhaled; FEV_1, forced expiratory flow in 1 s; FVC, forced vital capacity.

[a]Adjusted for maternal age, history of asthma and atopy, educational level, parity and psychological distress during pregnancy.

[b]Adjusted for maternal body mass index, smoking during pregnancy and pet keeping, and child's birth weight adjusted for gestational age, breastfeeding and daycare attendance.

[c]Adjusted for child's ethnicity.

[d]Adjusted for all confounders.

*P-value < .05.

**P-value < .01.

Bold values represent significant associations.

into non-significant (Table 3 and Table S2). The associations of the viruses with lung function were mainly explained by ethnicity of the child, while the association with inhalant allergic sensitization was explained by sociodemographic and health-related factors, lifestyle-related factors and ethnicity.

4 | DISCUSSION

4.1 | Principal findings

In this population-based prospective cohort study, we observed that seropositivity for EBV in early childhood was associated with higher lung function at school age. This association was driven by seropositivity for EBV in the presence of seropositivity for CMV. Seropositivity for EBV or CMV were not associated with asthma. Only seropositivity for CMV in the absence of seropositivity for EBV was associated with an increased risk of inhalant allergic sensitization. The associations of the viruses with respiratory health were fully explained by ethnicity, or sociodemographic and health-related factors, and lifestyle-related factors.

4.2 | Comparison with previous studies

We observed that EBV and CMV in early childhood were not independently associated with respiratory health in later childhood life. To our knowledge, this is the first study to associate EBV or CMV with lung function measures, and respiratory health later in childhood. A cross-sectional analysis within a prospective cohort study demonstrated that seropositivity for EBV was not associated with asthma or allergic sensitization at age 4 years.[2] Additionally, they demonstrated that CMV solely was not associated with asthma, allergy or allergic sensitization.[3] Two studies have shown that CMV in the absence of EBV was associated with an increased risk of allergic sensitization measured by IgE, at either 2 or 4 years of age.[3,4] Our result that CMV in the absence of EBV is associated with an increased risk of inhalant allergic sensitization is in line with these findings. However, we additionally found that these associations were explained by confounding factors. The composition of the study population, difference in confounders or measurement of allergic sensitization by skin prick test as opposed to specific serum IgE might explain this difference in results. We could not replicate the finding that EBV was associated with a decreased risk of allergic sensitization.[4] Similar to previous findings, we showed that EBV and CMV, solely or combined, were not associated with asthma.[2,3] A case-control study comparing CMV DNA between adults with and without asthma, demonstrated that those with asthma were more likely to be positive for CMV DNA.[25] Additionally, the number of CMV copies was higher in elderly, defined as age above 65 years, with asthma as compared to elderly without asthma. The number of CMV copies was not different in non-elderly with and without asthma. The number of CMV copies was also higher in elderly with asthma as compared to non-elderly with asthma. This suggests that age might be of importance, although due to the cross-sectional nature of the study it was not possible to disentangle the direction of the effect. Further studies are needed to examine the role of age of the child at virus infection, and the role of ethnicity and other confounding factors in the association of EBV and CMV with respiratory health across the life course.

4.3 | Strengths and limitations

This study was embedded in a population-based cohort study, and detailed measurements of respiratory health, and information on numerous confounders are important strengths. Some limitations should be discussed. First, prospective cohort studies have suggested that early infection with herpesviridae might lead to a decreased risk of atopy, measured by SPT or IgE, and late infections to an increased risk.[17,26] However, we measured seropositivity for IgG only and were not able to determine age of seroconversion. Additionally, children in our study with an infection occurring between 6 and 10 years of age would be classified as seronegative, leading to dilution of the effect. Second, it has been suggested that immune responses might differ between asymptomatic and mononucleosis-like infections. Unfortunately, we did not have detailed information on the clinical course of the herpesviridae infections. However, given that we study a childhood cohort with infections before 6 years of age, these infections are most likely mild or asymptomatic.[15] Third, information on asthma was obtained by questionnaire, which might have led to misclassification although a validated questionnaire was used to minimize bias. Last, selection bias due to loss to follow-up might have occurred. Most importantly, those lost to follow-up were more likely to be of non-European ethnicity, and more likely to be seropositive for EBV. This might have led to either an over- or underestimation of the effect, if the associations of those lost to follow-up with respiratory health would be different from those included in the study. This is unlikely, but cannot be excluded.[27,28]

4.4 | Underlying mechanisms

Some evidence on possible underlying mechanisms for the associations of herpesviridae with the immune and pulmonary system are provided in murine studies. One murine study demonstrated that mice infected in early life with murid herpesvirus 4, the murine equivalent of EBV, were protected against house-dust mite–induced airway allergy, compared to uninfected mice.[29] These infected mice had a reduced peribronchial infiltration by inflammatory cells, and lower numbers of eosinophils and interleukin-4. 5 and 6 in broncho-alveolar fluid. Additionally, the T-helper-2 polarization of house-dust mite–specific T cells, but not T-helper-1 polarization, was suggested to be impaired among infected mice. Another murine study found that after infection

with murid herpesvirus 4, or murine cytomegalovirus, more secretion of T-helper-1 cytokines was present, which might inhibit the T-helper-2 pathway.[30] In our study, we found that mainly ethnicity explained the association of EBV with a higher lung function. The association of CMV in the absence of EBV with inhalant allergic sensitization was explained by ethnicity, and also sociodemographic and health-related, and lifestyle factors. A previous study within our cohort demonstrated that non-Dutch children were more likely to be seropositive for EBV and CMV.[31] These ethnic differences were partly explained by socio-economic factors and factors related to crowding for EBV, but not for CMV. Other studies also demonstrated an association of ethnicity with the prevalence of herpesviridae, although the role of other factors explaining the association such as socio-economic or lifestyle-related factors in these studies were less prominent.[32,33] Another prospective cohort study demonstrated that maternal age was associated with time to infection for EBV and CMV, regardless of other factors such as daycare and siblings.[34] Additionally, they demonstrated that CMV is often acquired earlier than EBV, which might be explained by the immune response after CMV infection. Our findings suggest that there might be no causal association of EBV and CMV with respiratory health, but that ethnicity and possible other confounding factors are the underlying explaining mechanisms.

5 | CONCLUSION

Seropositivity for EBV and CMV at the age of 6 years were associated with a higher lung function and inhalant allergic sensitization, respectively, but not asthma at school age. However, the association of seropositivity for EBV with school-age lung function was explained by child's ethnicity, while the association of seropositivity for CMV in the absence of EBV with inhalant allergic sensitization was additionally explained by sociodemographic and health-related, and lifestyle-related factors. Future studies should focus on the role of age of the child at primary infection, specifically in relation to lung function and asthma, and the possible explaining role of ethnicity.

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CONFLICT OF INTERESTS

The authors declare no conflict of interest.

AUTHOR CONTRIBUTIONS

EM and LD contributed to the conception and design, acquisition of data, analyses and interpretation of the data, drafted the article, revised it critically for important intellectual content, and gave final approval of the version to be published. VJ, IR, MZ, JJ and HM contributed to the conception and design, acquisition of data, revised the drafted manuscript critically for important intellectual content, and gave final approval of the version to be published.

DATA AVAILABILITY STATEMENT

Research data are not shared.

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SUPPORTING INFORMATION

Additional supporting information may be found online in the Supporting Information section.

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