

# Herpesvirus infections and allergic sensitization in children of families with anthroposophic and non-anthroposophic lifestyle – the ALADDIN birth cohort

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## Keywords

Anthroposophy; cytomegalovirus; Epstein–Barr virus; HHV6; HHV7; IgE; infants; lifestyle; sensitization; viral infections

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**Background:** Growing up in families with an anthroposophic lifestyle has been associated with reduced risk of allergic disease in children. The aim of this report was to assess whether children with this lifestyle are infected earlier with Epstein–Barr virus (EBV), which has been associated with reduced risk of allergic disease, and three other herpesviruses potentially involved in allergy development, namely Human herpesvirus 6 (HHV6), Human herpesvirus 7 (HHV7) and cytomegalovirus (CMV).

**Methods:** Within the ALADDIN (Assessment of Lifestyle and Allergic Disease During Infancy), birth cohort study 157 children were categorized according to lifestyle into anthroposophic and non-anthroposophic. IgG-levels for EBV, HHV6, HHV7 and CMV were determined in plasma samples collected at ages 12 and 24 months and from parents. IgE levels against seven common allergens were analyzed at 24 months.

**Results:** No significant differences in seroprevalence of EBV, HHV7 or CMV were detected at any age between the two lifestyle groups. The seroprevalence of HHV6 was significantly lower in the anthroposophic group at 24 months of age (74.6% vs. 87.5%, *p*-value 0.048). Further, no significant associations between allergic sensitization and seropositivity to any of the viruses were detected; however, an interaction effect of lifestyle could not be ruled out.

**Conclusions:** Our results indicate that there is no strong influence of exposure to the anthroposophic lifestyle on the time for infection with EBV, HHV6, HHV7 or CMV. These infections can therefore not be assumed to be important factors in the allergy-protective effect of this lifestyle.

Lifestyle, like farming and anthroposophic lifestyle, has been shown to be potentially protective against allergic disease in childhood (1–4). The hygiene hypothesis suggests that infections in childhood, presumed to be related to lifestyle, influence the risk of development of allergic disease, but their role is complex and remains unclear (5).

Herpesviruses, such as Epstein–Barr virus (EBV), Human herpesvirus 6 (HHV6), Human herpesvirus 7 (HHV7) and cytomegalovirus (CMV), are of particular interest in the development of several diseases in humans because of their complicated interaction with the immune system with persistent expression of viral antigens and viral shedding after primary infection. Early EBV infection (before 2 years of age) has been associated with reduced risk of IgE-sensitization, an association that was stronger among

CMV-seropositive children, whereas later EBV-seroconversion rather seems to promote allergy development (6–8). The mechanism for this protective effect might be the polyclonal stimulatory effect of EBV on the immune system at a time when the child has not yet been exposed to many antigens and therefore lack allergen-specific, IgE-primed, B cells (9). Studies of CMV and HHV6 have been less conclusive about potential association to allergy development (6, 7, 10). HHV7 is a more recently discovered herpesvirus which is widespread among healthy children and whose relevance in allergy development has not been studied (11). The ways of transmission of these viruses differ from each other. EBV, HHV6 and HHV7 are mainly transmitted via saliva from family members, and CMV mainly via genital secretions at birth and breast milk.

However, seroprevalence (the proportion of seropositive individuals) in a population is dependent on lifestyle. In affluent countries, where allergic diseases are more prevalent, children seroconvert later to EBV and CMV than in non-affluent countries (12, 13). In an ongoing birth cohort study, ALADDIN (Assessment of Lifestyle and Allergic Disease During Infancy), the risk of IgE-sensitization to common allergens before 2 years of age, was reduced to one fourth among children of families with an anthroposophic lifestyle compared to children with conventional lifestyle (14). In many aspects, the anthroposophic lifestyle could be thought of as being less 'westernized'. The allergy-protective effect could hypothetically be attributed to several factors characteristic of this lifestyle, such as high proportion of home-births, restrictive use of antibiotics, antipyretics and vaccines, different intestinal microflora, prolonged breastfeeding, a stress-reducing environment, and later daycare attendance (14, 15).

In this study, we test our hypothesis that the lower allergy prevalence found among the children of families with anthroposophic lifestyle could be explained by earlier seroconversion to herpesviruses compared to other children.

Thus, the aim of this study was to compare the seroprevalence of EBV and three other herpesviruses at 12 and 24 months of age between children of families with anthroposophic and non-anthroposophic lifestyle.

## Methods

### The ALADDIN birth cohort

This study is part of the ALADDIN birth cohort, to which children were recruited before birth between September 2004 and November 2007 from anthroposophic and conventional Maternal Child Health Centres (MCHCs) in the Stockholm area. The study design, exposure characteristics, and allergic outcome of this cohort are previously thoroughly described by Stenius et al. (14). The study was approved by the Research Ethical Committee at Huddinge University Hospital, Stockholm, Sweden, and written informed consent was obtained from all parents.

### Categorization into lifestyle groups

Classification of the participating families into lifestyle groups was based on the choice of MCHCs and parental responses to three questions: (i) 'What kind of pre-school/school will your newborn child probably go to?', (ii) 'Has any of the parents, no matter which type of school you have planned for your child, an anthroposophic view of life?', and (iii) 'Is the family's everyday life influenced by an anthroposophic view of life?'. Families answering 'anthroposophic school' to question 1 and 'yes' to questions 2 and 3 and also attending anthroposophic MCHCs were defined as 'anthroposophic'. Families answering conventional or any other non-anthroposophic type of school to question 1, 'no' to questions 2 and 3, and going to conventional MCHCs were defined as 'non-anthroposophic'. Families with

any other combination of answers were not included in this study.

### Data collection

Demographic and exposure data were collected prospectively by questionnaires and interviews (14). Blood samples were obtained from the parents during pregnancy when the family was included in the study and from the child at 12 and 24 months of age. The samples were collected in heparin tubes and plasma stored at  $-20^{\circ}\text{C}$ .

### Virus serology

Serological analyses of EBV, HHV6, HHV7 and CMV were performed in plasma samples from children at 12 and 24 months of age and from parents in families where child samples from both ages were available. IgG against the EBV capsid antigen and HHV6 was determined according to previously published immunofluorescence assays (16, 17). IgG against HHV7 was determined by the same immunofluorescence method using antigen preparations from Scimedx Corporation (Denville, NJ, USA). A specific fluorescence in dilution of 1/20 was regarded as a sign of seropositivity. For CMV IgG detection, an Enzygnost<sup>®</sup> kit (Dade Behring, Marburg, Germany) was used according to the manufacturer's instructions, and a sample was considered positive if the absorbance was  $>0.2$  at a dilution of 1/100.

### Determination of IgE-sensitization

The plasma samples from the children at the age of 24 months were analyzed by ImmunoCAP<sup>®</sup> (Phadia AB, Uppsala, Sweden) for IgE to seven common allergens (hen's egg, cow's milk, peanut, cat, dog, birch and timothy) (14). A study subject was classified as sensitized if IgE levels were  $\geq 0.35$  kU/l for at least one of the seven allergens.

### Statistical analysis

Statistical analyses were conducted using IBM SPSS Statistics 20 software (Chicago, IL, USA). Fischer's exact test (for categorical variables) was used for the background variables and parental virus serology. Logistic regression was used for comparing results of the virus serologies of the children and odds ratios (ORs) and 95% confidence intervals (CIs) estimated. A  $p$ -value  $<0.05$ , two-sided, was regarded as statistically significant. A power calculation was performed for our main outcome, EBV seroprevalence at 24 months, and we assumed a seroprevalence of 25% in the reference population. Sample size calculations estimated that 60 children would be needed in each group to detect a risk ratio of 2.0 between the two groups, with a level of significance of 5%, two-sided, and a power of 80%.

A post hoc power calculation was performed with the actual sample sizes (88 and 59 children, respectively) and the actual EBV seroprevalence in the reference group (18.2%). This gives us 80% power to rule out a risk ratio of at least 2.2 between the two groups, given a significance level of 5%.

**Table 1** Demographic data on the 157 children and parental virus seroprevalence according to family lifestyle

|                            | Anthroposophic<br>n/N (%) | Non-anthroposophic<br>n/N (%) | p-value* |
|----------------------------|---------------------------|-------------------------------|----------|
| Female sex                 | 25/62 (40.3)              | 52/95 (54.7)                  | 0.10     |
| Older siblings             | 46/62 (74.2)              | 66/95 (69.5)                  | 0.59     |
| Breastfeeding              |                           |                               |          |
| at 6 months                | 56/62 (90.3)              | 71/95 (74.7)                  | 0.02     |
| at 12 months               | 29/61 (47.5)              | 23/92 (25.0)                  | <0.01    |
| Day-care at 18 months      | 16/62 (25.8)              | 62/94 (66.0)                  | <0.01    |
| Mother university-educated | 29/62 (46.8)              | 37/95 (38.9)                  | 0.41     |
| Father seropositive        |                           |                               |          |
| EBV                        | 42/46 (91.3)              | 69/76 (90.8)                  | 1.00     |
| HHV6                       | 38/46 (82.6)              | 62/76 (81.6)                  | 1.00     |
| HHV7                       | 45/46 (97.8)              | 76/76 (100)                   | 0.38     |
| CMV                        | 31/46 (67.4)              | 48/76 (63.2)                  | 0.70     |
| Mother seropositive        |                           |                               |          |
| EBV                        | 44/47 (93.6)              | 73/77 (94.8)                  | 1.00     |
| HHV6                       | 44/47 (93.6)              | 69/77 (89.6)                  | 0.53     |
| HHV7                       | 47/47 (100)               | 76/77 (98.7)                  | 1.00     |
| CMV                        | 29/47 (61.7)              | 48/77 (62.3)                  | 1.00     |

EBV, Epstein–Barr virus; HHV6, Human Herpesvirus 6; HHV7, Human Herpesvirus 7; CMV, cytomegalovirus.

\*Calculated by Fischer's exact test.

## Results

The recruitment process and flow of participants in ALAD-DIN, described in detail elsewhere (14), resulted in 181 families eligible for this report, 82 families belonging to anthroposophic,

and 99 families to the non-anthroposophic lifestyle group. Of these families, 24 could not be included due to no available child plasma, 20 (24%) from the anthroposophic, and 4 (4%) from the non-anthroposophic group. Among the remaining 157 families, plasma was available from 140 children at the age of 12 months (53 in the anthroposophic and 87 in the non-anthroposophic group) and from 147 children at the age of 24 months (59 in the anthroposophic and 88 in the non-anthroposophic group). From 50 (60.1%) children in the anthroposophic and 80 (80.1%) in the non-anthroposophic group, blood was available from both ages, and in these families, plasma from 124 mothers and 122 fathers was analyzed.

In Table 1, demographic data, hypothetically related to herpesvirus exposure, for the 157 children are presented. In the anthroposophic group, a significantly higher proportion of the children were breastfed at six and twelve months of age and significantly fewer children attended day-care at 18 months. There were no significant differences in the proportions of children having older siblings or university-educated mother between the two lifestyle groups. The seroprevalences of the four herpesviruses among the parents were similar in the two lifestyle groups.

The seroprevalence of EBV, HHV6, and HHV7 among the children increased markedly between 12 and 24 months of age, that of HHV6 being highest at both ages (54.3% and 82.3% respectively). CMV seroprevalence remained relatively unchanged (23.6% and 26.5%, respectively), indicating that CMV is less commonly acquired in this time window. No significant differences in the proportions of children seropositive to EBV, HHV7, and CMV were detected at any age between the two lifestyle groups. The proportion of HHV6 seropositive children was significantly lower in the anthroposophic group at 24 months of age, 74.6% vs. 87.5%, OR 0.42 (0.18–0.99). Among the 130 children where plasma was

**Table 2** Seroprevalence of four herpesviruses in children at 12 and 24 months of age and comparison between the two lifestyle groups

| Virus                                   | All<br>n/N (%) | Anthroposophic<br>n/N (%) | Non-anthroposophic<br>n/N (%) | p-value* | OR* (95% CI)     |
|-----------------------------------------|----------------|---------------------------|-------------------------------|----------|------------------|
| 12 months                               |                |                           |                               |          |                  |
| EBV                                     | 10/140 (7.1)   | 4/53 (7.5)                | 6/87 (6.9)                    | 0.88     | 1.10 (0.30–4.10) |
| HHV6                                    | 76/140 (54.3)  | 27/53 (50.9)              | 49/87 (56.3)                  | 0.54     | 0.81 (0.41–1.60) |
| HHV7                                    | 16/140 (11.4)  | 6/53 (11.3)               | 10/87 (11.5)                  | 0.98     | 0.98 (0.34–2.88) |
| CMV                                     | 33/140 (23.6)  | 11/53 (20.8)              | 22/87 (25.3)                  | 0.54     | 0.77 (0.34–1.76) |
| 24 months                               |                |                           |                               |          |                  |
| EBV                                     | 27/147 (18.3)  | 11/59 (18.6)              | 16/88 (18.2)                  | 0.94     | 1.03 (0.44–2.41) |
| HHV6                                    | 121/147 (82.3) | 44/59 (74.6)              | 77/88 (87.5)                  | 0.048    | 0.42 (0.18–0.99) |
| HHV7                                    | 52/147 (35.4)  | 22/59 (37.3)              | 30/88 (34.1)                  | 0.69     | 1.15 (0.58–2.29) |
| CMV                                     | 39/147 (26.5)  | 12/59 (20.3)              | 27/88 (30.7)                  | 0.17     | 0.58 (0.27–1.26) |
| Seroconversion between 12 and 24 months |                |                           |                               |          |                  |
| EBV                                     | 15/130 (11.5)  | 6/50 (12.0)               | 9/80 (11.2)                   | 0.90     | 1.08 (0.36–3.23) |
| HHV6                                    | 38/130 (29.2)  | 13/50 (26.0)              | 25/80 (31.2)                  | 0.52     | 0.77 (0.35–1.70) |
| HHV7                                    | 30/130 (23.1)  | 10/50 (20.0)              | 20/80 (25.0)                  | 0.51     | 0.75 (0.31–1.77) |
| CMV                                     | 2/130 (1.5)    | 0/50 (0.0)                | 2/80 (2.5)                    | –        | –                |

EBV, Epstein–Barr virus; HHV6, Human Herpesvirus 6; HHV7, Human Herpesvirus 7; CMV, cytomegalovirus.

\*Calculated by logistic regression. Non-anthroposophic group reference group.

**Table 3** Seroprevalence for four herpesviruses at 12 and 24 months of age and IgE-sensitization at 24 months of age

|                                         |               | Total | IgE-sensitized<br>at 24 months (%)* | OR (95% CI)†     | p-value |
|-----------------------------------------|---------------|-------|-------------------------------------|------------------|---------|
| Virus serostatus at 12 months           |               |       |                                     |                  |         |
| EBV                                     | –             | 131   | 32 (24.4)                           |                  |         |
|                                         | +             | 122   | 31 (25.4)                           | 0.37 (0.04–3.05) | 0.35    |
| HHV6                                    | –             | 9     | 1 (11.1)                            |                  |         |
|                                         | +             | 61    | 16 (26.2)                           | 0.83 (0.38–1.85) | 0.65    |
| HHV7                                    | –             | 70    | 16 (22.9)                           |                  |         |
|                                         | +             | 116   | 28 (24.1)                           | 1.14 (0.34–3.87) | 0.83    |
| CMV                                     | –             | 15    | 4 (26.7)                            |                  |         |
|                                         | +             | 99    | 24 (24.2)                           | 1.04 (0.41–2.62) | 0.93    |
| Virus serostatus at 24 months           |               |       |                                     |                  |         |
| EBV                                     | –             | 147   | 33 (22.4)                           |                  |         |
|                                         | +             | 120   | 29 (24.2)                           | 0.55 (0.17–1.71) | 0.30    |
| HHV6                                    | –             | 27    | 4 (14.8)                            |                  |         |
|                                         | +             | 26    | 5 (19.2)                            | 1.27 (0.44–3.66) | 0.67    |
| HHV7                                    | –             | 121   | 28 (23.1)                           |                  |         |
|                                         | +             | 95    | 22 (23.2)                           | 0.89 (0.39–2.02) | 0.78    |
| CMV                                     | –             | 52    | 11 (21.2)                           |                  |         |
|                                         | +             | 108   | 24 (22.2)                           | 1.05 (0.44–2.51) | 0.91    |
| Seroconversion between 12 and 24 months |               |       |                                     |                  |         |
| EBV                                     | Unchanged     | 130   | 32 (24.6)                           |                  |         |
|                                         | Seroconverted | 115   | 30 (26.1)                           | 0.44 (0.09–2.05) | 0.29    |
| HHV6                                    | Unchanged     | 15    | 2 (13.3)                            |                  |         |
|                                         | Seroconverted | 92    | 20 (21.7)                           | 1.66 (0.71–3.87) | 0.24    |
| HHV7                                    | Unchanged     | 38    | 12 (31.6)                           |                  |         |
|                                         | Seroconverted | 100   | 25 (25.0)                           | 0.91 (0.35–2.38) | 0.85    |
| CMV                                     | Unchanged     | 30    | 7 (23.3)                            |                  |         |
|                                         | Seroconverted | 128   | 31 (24.2)                           | –                | –       |
|                                         |               | 2     | 1 (50.0)                            |                  |         |

EBV, Epstein–Barr virus; HHV6, Human Herpesvirus 6; HHV7, Human Herpesvirus 7; CMV, cytomegalovirus.

\*Defined as having specific IgE > 0.35 kU/l for at least one of the investigated allergens (milk, hen's egg, cat, dog, timothy, birch, or peanut) in plasma sample at 24 months of age, % of total.

†Odds ratio for being IgE-sensitized in the seropositive (or seroconverted) group compared with the seronegative (or unchanged) group calculated by logistic regression. Non-seroconverted reference.

available from both 12 and 24 months of age, the proportions of children who seroconverted between the two sampling occasions were calculated for the different viruses. Only two of these children (1.5%) acquired CMV between 1 and 2 years of age (Table 2).

In addition, the available data on allergic sensitization among these children were used to analyze the association between virus seropositivity at 12 and 24 months and IgE-sensitization at 24 months of age. For HHV6-, HHV7-, and CMV-seropositivity, odds ratios for being IgE-sensitized at 24 months ranged from 0.83 to 1.27 with p-values ranging from 0.65 to 0.93, whereas for EBV-seropositivity, the corresponding odds ratios and p-values were lower, although not statistically significant (Table 3).

To test for effect modification of lifestyle on the association between EBV-seropositivity at 24 months and IgE-sensitization, the interaction between EBV and lifestyle was included in the logistic regression model (p-value 0.24). The odds ratio for being sensitized at 24 months comparing the EBV-seropositive with the EBV-seronegative children was 1.30 (0.23–7.34),

p-value 0.77, in the anthroposophic group and 0.32 (0.07–1.55), p-value 0.16, in the non-anthroposophic group.

## Discussion

This study could not confirm the hypothesis that children in families with anthroposophic lifestyle seroconvert earlier to EBV, HHV6, HHV7, or CMV. Instead, our results indicate that there is no strong influence of this lifestyle on the timing of seroconversion for these viruses. Opposite to our hypothesis, we observed that the anthroposophic children had a lower seroprevalence for HHV6 at 24 months. Although an association between early HHV6 infection and reduced risk of IgE-sensitization was seen in another small cohort recently (10), our results do not indicate such an association, and HHV6-infection might not be an important factor in allergy development.

In addition to the primary aim of this study, we investigated the association between seropositivity of these four viruses and IgE-sensitization at 24 months of age. No conclusions can be drawn from these data because of the small numbers, but our

data support previous findings that early EBV infection seems to be associated with reduced risk of IgE-sensitization. Interestingly, signs of such association can only be seen among the non-anthroposophic children, among whom the risk-estimate (odds ratio, 0.32) in fact is similar to that observed in a different cohort previously (6). This could hypothetically indicate that children who are already protected against allergy by the anthroposophic lifestyle might not get additional protective effect of early EBV infection.

The seroprevalences for the different viruses among the non-anthroposophic children and parents are in accordance with what is expected in a population with high socioeconomic status in the western world (6, 13, 18, 19); therefore, we can consider the non-anthroposophic group being representative controls.

One limitation of the study is the rather low number of participants. However, the primary aim of this study was neither to completely exclude any influence of anthroposophic lifestyle on the seroprevalence of these viruses, nor to investigate the role of these early infections in allergy development. Our aim was to test our hypothesis that earlier seroconversion could be one major explaining factor to the markedly reduced allergy prevalence among children in families with this lifestyle, which is why the difference in EBV seroprevalence was expected to be at least twofold between the two groups to be considered clinically significant. One should also consider that the proportion of children where plasma was available was

smaller in the anthroposophic group at both ages due to greater reluctance among the parents in this group to agree to blood sampling of the child. On the other hand, in this same cohort of children, exposure to the anthroposophic lifestyle, defined the same way, was associated with reduction of the risk of IgE-sensitization the first 2 years of life to one fourth compared to being unexposed (14). A lifestyle factor explaining this allergy-protective effect is, therefore, expected to be differentially distributed between the two groups.

In conclusion, infections with EBV, HHV6, HHV7, and CMV do not seem to be an important part of the explanation for the reduced allergy development in children of families with an anthroposophic lifestyle, but an interaction effect of lifestyle and EBV-seroconversion on allergic sensitization could not be ruled out.

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