

Correlation between Epstein Barr virus antibodies, serum IgE and atopic disease

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It is currently accepted that viral infections may influence the development of atopy. In the present study we evaluated serum IgE levels as well as the prevalence of symptoms indicative of atopic disease and EBV antibodies in 353 children aged from 1 month to 19 years. Antibodies against EBV were detected by immunofluorescence. IgE levels in serum were measured by enzyme immunoassay. Dividing the study population according to EBV seropositivity and age, we noted that the prevalence of high IgE levels (> 2 s.d.) was, in total, more frequent in the EBV negative (32.9%) than in the positive subjects (27.6%). Interestingly, this higher prevalence was found only in the groups aged under six, especially in the 7 to 29 month group, where it was statistically significant ($p=0.037$), whereas in the 6–19 year group the situation was reversed. Furthermore, selecting only the atopic children younger than 3 years of age with high IgE levels and clinical symptoms of atopy (wheezing and/or dermatitis) it was possible to demonstrate lower EBV seropositivity compared with the normal IgE controls for each group, even though these differences were not statistically significant. In conclusion, the results of our study suggest that, in our selected population, EBV infection in the first years of life is associated with a lower prevalence of high IgE levels.

Abbreviation: EBV: Epstein Barr virus.

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It is currently accepted that both genetic and environmental factors may influence the development of atopy. Of the latter, infections seem to have great importance, especially those occurring in the early years of life (1). Respiratory viral infections, for instance, may induce the development of allergic respiratory diseases causing damage to the airway tissue and inducing an IgE response to inhalants (2). Epstein Barr virus (EBV), has also been suspected of being able to cause immunologic changes resulting in the development of atopy (3). In contrast, recent epidemiological findings have suggested that viral infections early in life may prevent allergic sensitization and the development of atopy (4–5).

In the present study we evaluated serum IgE levels as well as the prevalence of symptoms indicative of atopic disease and EBV antibodies in 353 children who were hospitalized in the Pediatric Department of St. Camillo Hospital (Rome).

Materials and methods

The medical records of 353 children (185 boys and 168 girls) aged from 1 month to 19 years (average 4 years) were studied retrospectively in order to evaluate the association between atopy and EBV infection. Of these children, 223 were under 3 years of age and 130 were older. They were selected from a group of 1,123 children consecutively hospitalized during the years 1990–1992 and enrolled in a previous clinical and epidemiological study on Epstein Barr virus (EBV) infection (6). Regardless of the diagnosis, EBV IgG and IgM antibodies against Viral Capside Antigen (VCA) were assayed in the children admitted during this two year period, since blood samples were available.

The diagnoses on admission were mainly of febrile respiratory diseases, often with wheezing, but also acute and chronic bowel diseases, urinary tract

infections, convulsive crises and food intolerance were diagnosed. All the children were examined by at least one of the authors.

Antibodies against EBV were detected by indirect immunofluorescence that measured IgG and IgM antibodies to VCA. The presence of antibodies was determined at a minimum dilution of 1:20 with further dilutions in some samples up to the end titer. A VCA IgG titer greater or equal to 1:20 without VCA IgM was considered indicative of previous infection. Each test run was carried out according to the producer's instructions and with positive and negative controls at the screening dilution of 1:20. The features of specificity, sensitivity and reproducibility of the assay were regularly checked by end-point titration of controlled pools of sera, performed as quality control tests in our laboratory. We used the 1:20 screening dilution because it was shown to increase assay specificity without any significant reduction in sensitivity.

We included in the present study all 353 records (of a total of 1,123) for which both total IgE and IgG and IgM VCA results were available.

Total IgE levels were measured on admission in those patients showing either atopic symptoms or having a family or personal history which indicated possible atopy. They were also measured in order to evaluate immune function.

IgE levels in serum were measured by enzyme immunoassay (Kallestad Pasteur), performed according to the standard reference WHO 75/502 (7). IgE values were expressed in UI/ml. The study considered as atopic IgE levels greater than 2 s.d. of the normal values/age in the general Italian population. (8) The remaining children were used as controls.

Allergic diseases were defined as follows:

Wheezing: children with expiratory wheezes and coughing during hospitalization who required bronchodilator therapy and/or who had already shown these symptoms several times and who had already received this therapy;

Allergic Dermatitis: children affected by atopic dermatitis or rash considered as having an allergic origin on the basis of medical history and laboratory tests (rast, prick tests). We excluded children with acute EBV infection (IgM VCA $\geq$ to 1:20) or with immune deficiency, collagenosis, autoimmune diseases, parassitosis and children who had recently received corticosteroid therapy (os, im, iv).

Statistical analysis

The computerized program Epi Info 5.01 was used for statistical analysis (9).

Statistical significance was determined by chi-square and where necessary by Fisher's exact test

Table 1. Prevalence of EBV seropositivity according to age

Age Group	1-2 months	3-5 months	6-12 months	13-24 months	25-36 months	3-6 years	7-19 years
n	(9)	(35)	(64)	(72)	(43)	(48)	(82)
EBV + (%)	88.8	57.1	29.6	36.1	41.8	47.9	76.8

and Mantel-Haenszel Summary Chi Square. A p value < 0.05 was considered to be significant.

Results

An antibody titer showing a previous EBV infection was found in 177/353 (50.1%) children (100 males and 77 females) (p=ns). The EBV seropositive percentage dropped from 88.8% of the 1-2 month group to 29.6% of the 6-12 month group, probably due to the presence of maternal antibodies. This increased to 76.8% in the children over 6 years of age (Table 1).

High IgE levels (> 2 s.d.) were found in 107/352 children (30.3%). Of these 72 were male, 35 were female (M/F = 2.05:1). This difference, already observed by others, was statistically significant. (p=0.0002) (10).

Dividing the population according to EBV seropositivity and age, we noted that the prevalence of high IgE levels was, in total, more frequent in the EBV negative children (32.9%) than in the positive subjects (27.6%), even though the difference was not statistically significant (p=0.55).

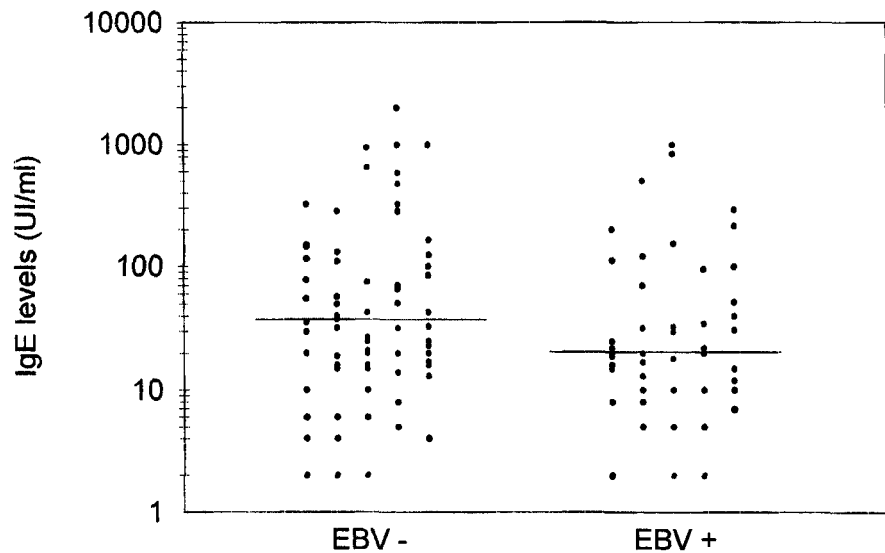
It is interesting to note that this higher prevalence was found only in the range from the 1-5 month group to the 3-6 year group, whereas in the 7-19 year group the situation was reversed.

In order to exclude any significant maternal antibody interference, we selected a group of 149 children, subgroup A, aged over 6 months. In this sub-

Table 2. Prevalence of high IgE levels in the EBV seropositive and seronegative groups according to age. Lower prevalence is statistically significant in the EBV seropositive 7-29 month group compared to EBV seronegative (p=0.037)

AGE	EBV + n	IgE>2 s.d. (%)	EBV - n	IgE>2 s.d. (%)	p
1-5 months	28	17.8	16	25	-
6-12 months	19	26.3	45	40	-
13-24 months	26	11.5	46	23.9	-
25-36 months	18	44.4	25	48	-
3-6 years	23	17.3	25	24	-
7-19 years	63	38	19	36.8	-
1 month-19 years	177	27.6	176	32.9	-
7-29 months (subgroup A)	50	18	99	34.3	0.037

Fig. 1. Levels of IgE by EBV seropositivity in the subgroup A (7–29 months group). Horizontal bars indicate geometric mean titers.



group (age range 7 to 29 months) we found the prevalence of high IgE levels was statistically more frequent in the EBV negative children (34.3%) than in the positive subjects (18%) ($p=0.037$) (Table 2).

This difference was still statistically significant even after stratifying the population according to sex (Mantel-Haenszel Summary Chi Square = 4.16, $p=0.041$).

The distribution of IgE levels by EBV seropositivity in the subgroup A is shown in Fig. 1. The geometric mean total IgE was higher (37.04 UI/ml) in EBV negative than in EBV positive (23.74 UI/ml).

Our study went on to evaluate EBV seropositivity in children affected by wheezing and/or atopic dermatitis and with high IgE levels. Also in this subgroup of 45 children, subgroup B, all aged under 3 years, it was possible to demonstrate lower EBV seropositivity compared with the normal IgE controls, even though these differences were not statistically significant. EBV seropositivity was detected in 16/45 (35.5%) atopics versus 79/157 (44.5%) non atopics ($p=0.08$) whereas in the 7–29 month age band

EBV seropositivity was detected in 7/25 (28%) atopics versus 41/106 (38.6%) non atopics ($p=0.31$) (Table 3).

The relatively high percentage of symptoms such as wheezing or allergic dermatitis in the control group is due to the fact that our study population had previously been selected because most of them showed some symptoms or had a family or personal history indicating possible atopy.

Discussion

The relationship between viral infections and atopia is complex and still to be clarified. Several observations would indicate that viral infections can favour the development of atopia. For example, it is now accepted that certain respiratory viruses, mainly respiratory syncytial virus, parainfluenza virus and rhinovirus are important trigger factors in the development of allergic respiratory diseases, particularly asthma (11–13). However, EBV doesn't seem to have this kind of impact. In fact, primary EBV in-

Table 3. Serum IgE levels (mean), prevalence of wheezing and of allergic dermatitis in the atopics and non atopics groups. Prevalence of positive EBV VCA antibodies is lower in the atopics than in the non atopics groups

Months	ATOPICS					CONTROLS				
	n	IgE (mean)	AD (%)	WHEEZING (%)	EBV+ (%)	n	IgE (mean)	AD (%)	WHEEZING (%)	EBV+ (%)
1–5	8	141	62.5	37.5	50	35	6	17.1	42.8	65.7
6–12	16	222	37.5	62.5	31.2	41	12	12.1	51.2	34.1
13–24	8	322	37.5	62.5	37.5	58	26	22.4	31	39.6
25–36	13	533	30.7	76.9	30.7	23	32	21.7	43.4	43.4
7–29	25	380	36	64	28	106	22	9.4	36.7	38.6
1–36 (subgroup B)	45	306	46.6	62.2	35.5	157	19	18.4	41.4	44.5

fection is generally asymptomatic in the first 2–3 years of life or may induce mild clinical symptoms (14), which are only partly characteristic (6). Infection after this age, however, leads to a clinical picture ever more typically that of mononucleosis (15).

Less well known is the relationship between viral infections and the development of allergic sensitization. Certain recent observations based on *in vitro* models and animal experiments suggest that viruses or viral infections may promote IgE sensitization (16–18).

A 4-year prospective study of 13 atopic prone infants conducted by Frick et al. showed a temporal association in 10 of the 11 children who subsequently become atopic between viral respiratory tract infections and the onset of respiratory allergic symptoms (19).

In contrast, it has recently been suggested that viral infections during the first years of life may actually hamper the development of atopy tipping the balance in favour of TH-1 like helper cells, thereby inhibiting the synthesis of IgE (20). Evidence to support this hypothesis comes from epidemiological studies showing a decrease in the prevalence of atopic sensitization with increasing numbers of siblings (21), with greater numbers of households living together (5) or with a decline in socioeconomic conditions (22). Such situations are becoming less frequent in industrialized countries. This decrease in the frequency of infection in the first years of life and/or the postponement of infection beyond a certain critical age could explain the progressive increase in atopic diseases in industrially advanced nations (23).

Of the viruses thought to play a role in the development of atopy, EBV is particularly worthy of attention. It is widely diffused in the world and is well correlated with the epidemiology of atopy. EBV infection mainly takes place in the first years of life and it is more common and precocious in underdeveloped countries where 80–90% of 2–3 years old children are positive for antibody titer (24). In industrialized countries the infection takes place much later on and only 50% of 5 year old children show a significant antibody titer (25).

EBV selectively infects B lymphocytes via binding of the major virus envelope protein to CD21, the receptor for the C3d,g and iC3b proteins of the complement system (CR2). However CD21 is also the receptor for interferon- α and CD23, a low affinity receptor for human IgE (FC ϵ R2) (26).

It has been demonstrated that CD23 plays a role in the regulation of IgE synthesis *in vitro* (27) and *in vivo* (28) by binding to CD21 on B cells. An interesting recent study has shown that EBV is able to inhibit this binding in a dose dependent manner (29).

Finally, *in vitro* studies have demonstrated that two signals are required for the induction of IgE synthesis in human B-cells.(30). The first is delivered by IL-4 (31) the second by a series of B cell activators such as contact dependent T- and B-cell interaction or CD40/CD40 ligand, glucocorticoids (32) but also transformation with EBV (33).

Thus EBV could theoretically act both positively and negatively in the development of atopy.

Other authors have previously reported an association between EBV infection, serum IgE levels and diseases mediated by IgE.

Nordbring et al. (34) and then Bahna et al. described an elevation of serum IgE during acute EBV infection (35) although this was not confirmed in a more recent study (36). Olson and coworkers showed a high frequency of IgE mediated allergic disease in “chronic mononucleosis syndrome” (37).

Moreover, there are at least two studies in which an association was sought between the prevalence of atopy and EBV seroconversion. In the first, Strannegard found that the frequency of EBV seropositivity and antibody titers were higher in atopic than in non atopic children, even though this difference was statistically significant only for antibody titers (38) This observation was confirmed by the second study carried out by Rystedt et al. on a larger number of adults with atopic dermatitis in which both frequency of EBV seropositivity and antibody titers were found to be statistically significant (3).

On the basis of all these observations, and since EBV selectively infects B lymphocytes causing a polyclonal activation with consequent lymphocyte hyperproduction of immunoglobulins (especially IgM and IgE), Rystedt et al. suggested that EBV infection in atopic individuals may result in IgE response to any antigen and in this way might precipitate atopic diseases (3).

At first sight the results of our study showing greater prevalence of high IgE levels in EBV negative children appear to be in disagreement with what has previously been observed by others. However, we must take into account the fact that all the studies we have mentioned before were carried out on children over 5 years of age or on adults. Strannegard's study showing greater seropositive prevalence for EBV in atopic subjects, was conducted on a group of 137 children aged between 5 to 18 years, while Rystedt's study was carried out on 24–44 year old patients. Also in our study population, the frequency of atopics was higher in the EBV seropositive group aged over seven years (38% vs 36.8%) (Table 2). Yet, if we consider the entire group or only the groups representing the first years of life, the ratio is reversed.

The observation of lower IgE levels in EBV positive children support the hypothesis that infections

in the first years of life may hamper the development of atopy.

It is not clear whether lower IgE levels in EBV positive children can be caused by upregulation of TH-1 like helper cells, given that IFN γ levels, for example, have been reported normal (36), increased (39) and decreased (40) in studies on EBV infection.

On the other hand Bahna demonstrated that in the course of primary EBV infection, IgE levels usually rise to a peak during the first week of illness and then fall rapidly to below pre-illness levels for several months, gradually returning to pre-illness levels over a period of about one year (35). This rebound effect could explain the lower levels of IgE found in EBV positive children in the first years of life. In fact it is highly probable that the percentage of subjects showing this rebound effect will be much higher in younger age groups, where infection is likely to be recent, rather than in older age groups. In this case the inhibiting effect on IgE synthesis would only be temporary. However, this would still be noteworthy since it is known that high IgE levels during infancy are predictive of subsequent development of atopy (41–42). Furthermore it has recently been hypothesized that IgE can play a role “in allergy expansion by generating allergies to new allergens” (43).

Our retrospective observational study was carried out on a selected population and caution is required in extrapolating the results to the general population. In addition, further studies are required in order to take into account the other factors that determine serum IgE.

In conclusion, the results of our study are not consistent with the Rystedt hypothesis that EBV infection might precipitate atopic disease. On the contrary, they suggest that EBV infection, if contracted in the first years of life, is associated with a lower prevalence of high levels of IgE.

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