

Respiratory syncytial virus infection: its role in aeroallergen sensitization during the first two years of life

Forster J, Tacke U, Krebs H, Streckert HJ, Werchau H, Bergmann RL, Schulz J, Lau S, Wahn U. Respiratory syncytial virus infection: its role in aeroallergen sensitization during the first two years of life. *Pediatr Allergy Immunol* 1996; 7: 55–60. © Munksgaard 1996.

Our aim was to study the influence of infection with the respiratory syncytial virus (RSV) in non-hospitalized infants on sensitization to aeroallergens and the early manifestation of atopy. Six hundred and nine infants from the prospective German Multicenter Cohort Study on Atopy were included, 38% of whom had an elevated atopic risk. RSV IgG and IgM antibodies were tested by ELISA with gradient purified RSV antigen. Specific IgE against mites, cat dandruff, birch and grass pollens and relevant nutritional antigens were tested with CAP-RAST-FEIA (Pharmacia, Sweden). Of the cord sera 99% were positive for RSV-IgG, 44.7% at one year and 64.2% (n=265) at two years of age. The positivity rate after 12 months varied with the season of birth, the number of siblings and the degree of exposure to tobacco smoke; and correlated closely with attacks of wheezing during infancy. Twenty (2.8%) children were found to be sensitized against at least one aeroallergen at one year, and 28 (10.5%) at two years. By the first birthday, mite sensitization (n=3) could only be seen in the RSV-infected children; grass pollen sensitization (n=9) was associated with RSV seropositivity (logistic regression model including the confounders mentioned above: with RSV IgG $p=0.048$ and IgM $p=0.0006$), as was birch sensitization (n=5) with RSV IgM ($p=0.009$). No such differences could be detected at two years. No correlation of RSV seropositivity to any allergic manifestation could be found. We conclude, that it is only in the first year of life, that RSV infection plays a significant role in promoting sensitization against aeroallergens, which do not at this time produce allergic symptoms.

**J. Forster¹, U. Tacke¹, H. Krebs²,
H. J. Streckert², H. Werchau²,
R. L. Bergmann³, J. Schulz³, S. Lau³,
U. Wahn³**

Departments of Pediatrics ¹University Hospital, Freiburg, ²Rudolf Virchow University Hospital, Berlin, ³Institute for Clinical Microbiology and Virology, Ruhr University, Bochum, Germany

Johannes Forster, University Children's Hospital, Mathildenstr. 1, D-79106 Freiburg, Germany

Key words: allergic sensitization; aeroallergens; infant; respiratory syncytial virus

Received December 30, 1995

Accepted February 27, 1996

In animal studies, RSV has been shown to promote allergic sensitization (1–3), and in infancy it is the most important cause of wheezing and bronchiolitis. It has been suggested that it may trigger bronchial obstruction and sensitization against aeroallergens up to three years of age (4) and cause asthma-like symptoms by inducing virus specific IgE-antibodies (5), or trigger allergic symptoms in children at high risk for atopy because of their genetic disposition. This latter idea is derived from old reports describing the incidence of the symptoms of hay fever after virus-like illnesses in children (6). Later, as a result of small prospective studies with continuous monitoring for viral antigens, the association of viral respiratory tract infection with sensitization against aeroallergens was described (summarized in (7)).

Conflicting data emerged last year from a large prospective study which showed that multiple respiratory tract infections in infancy and childhood are associated with a reduction in the prevalence of allergic sensitization in schoolchildren (8).

However, there has so far been no prospective study to decide whether RSV, the clinically most important respiratory tract virus in infancy, promotes allergic sensitization against aeroallergens or atopic manifestations in general.

We took advantage of the German Multicenter Atopy Study (MAS 90), initially designed to monitor the predictive value of various clinical and immunological parameters (including the known cofactors affecting the manifestation of atopy in infancy and early childhood), to answer the following questions:

1. Is RSV infection associated with early sensitization against aeroallergens up to the age of 24 months?
2. Are atopic manifestations (eczema, food allergy, rhinoconjunctivitis) associated with early RSV infection?

Participants in the study

Between January 1 and December 31 1990, 7609 full-term newborns were screened for participation in the prospective German Multicenter Atopy Study (9). The group at risk for atopic manifestations included all infants with at least two atopic members in the immediate family circle and/or cord-blood containing IgE ≥ 0.9 kU/l (CAP-FEIA, Pharmacia, Sweden) ($n=385$). Controls were randomly selected from other newborns in the proportion 2:1, so that eventually 35.7% of the participants belonged to the high risk group. Informed written consent to participation was obtained from 1314 families. The present study is a report on 609 individuals of whom not only cord serum was available together with a complete dataset of the clinical variables, but also a serum taken at 12 months of age. These eligibility criteria did not alter the socioeconomic and atopic risk characteristics of the sample. Neither were they statistically different in the 265 participants of whom we obtained a serum at 24 months.

Procedure

The infants were seen in the six pediatric outpatient clinics participating at 1, 3, 6, 12, 18, and 24 months after birth. At each visit, data on the atopic symptoms of the infant and its family, infantile diseases, nutrition, development and behavior were obtained by questionnaires, and from a structured interview. In addition, data on environmental factors such as exposure to tobacco smoking and household pets, home conditions (size of house or apartment, rural or urban location, floor coverings, etc.) and other relevant sociodemographic factors were collected.

At each visit a structured clinical 'well baby' examination was carried out by the study physician. Great attention was paid to atopic signs, especially skin manifestation. Depending on the diagnosis of the family physician, the findings of the study doctor and the parents' reports of relevant atopic symptoms, each child was placed into one of three groups: 'not', 'probably' or 'obviously' atopic. Obvious asthma required at least one of the following features: (1) the diagnosis of wheezing bronchitis by the family doctor, reported by the parents; (2) symptoms of wheezing more than twice since the last visit; (3) diagnosis of obvious wheezing bronchitis by the study doctor, due to the reported symptoms; (4)

diagnosis by the study doctor, due to the physical examination. Probable asthma required at least one of the following features: (1) symptoms of wheezing less than three times since the last visit; (2) diagnosis of probable wheezing due to the reported symptoms; (3) diagnosis of probable wheezing due to the physical examination.

'Obvious atopic eczema' required a combination of three or more characteristic morphological features at three typical locations (9).

IgE antibodies

The total IgE was measured in kU/l with CAP-RAST-FEIA (Pharmacia, Sweden). Sensitization was defined as the presence of specific IgE-antibodies against at least one of the 8 major allergens (house dust mite, cat dandruff, birch and grass pollens, milk, chicken, wheat and soy in excess of 0.35 kU/l).

RSV antibodies

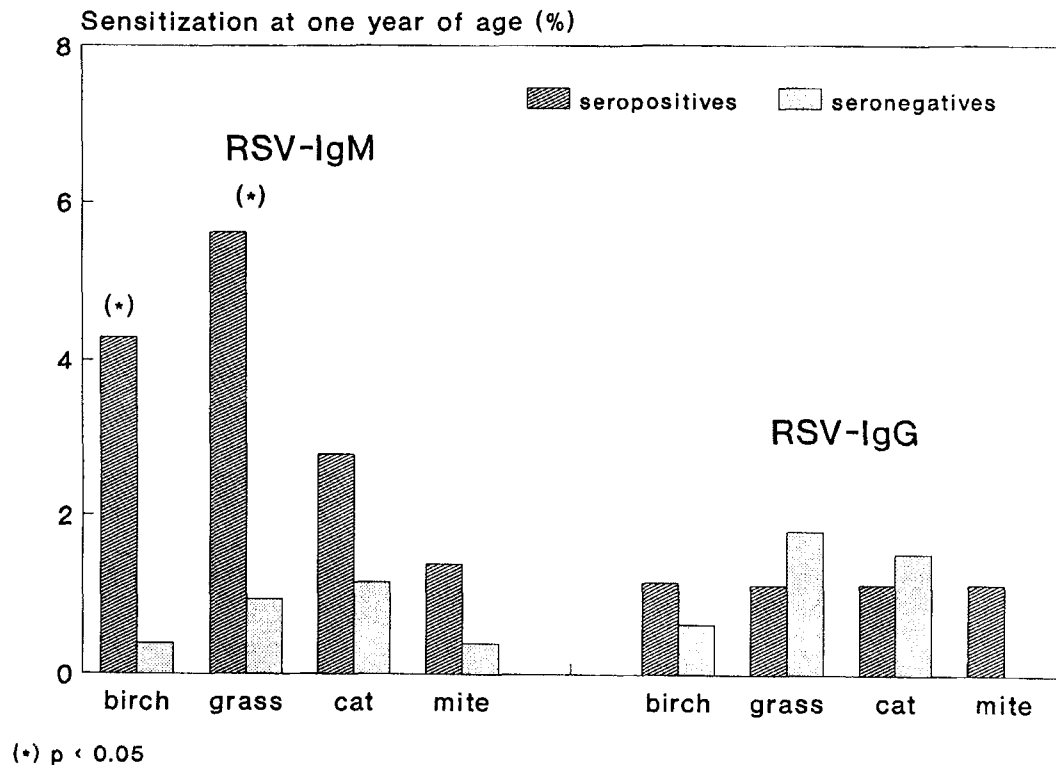
RSV-antibodies were determined by a sandwich-type ELISA. As the antigen, 110 ng of density gradient purified RSV in 75 μ l of 0.5 M bicarbonate buffer (pH 9.6) was added to each well of an ELISA plate (Maxisorb, Nunc, Wiesbaden, Germany) (10). After incubation for 1 hour at room temperature, the plates were kept at 4°C overnight. The unbound RSV proteins were washed off in two steps with PBS + 0.05% Tween 20 buffer. Incubation at room temperature with the same buffer was carried out for 1 hour to reduce nonspecific binding. 75 μ l of 1:100 diluted serum was added to each well. After 1 hour incubation at 37°C, the unbound antibodies were washed off. The immunocomplexes were labeled for 1 hour with an alkaline phosphatase conjugated anti-human antibody at the appropriate dilution (Sigma, Deisenhofen, Germany). P-phenylphosphate was used as a substrate. After 1 hour, the color reaction was stopped with 2 N sulfuric acid and absorption determined at 405 nm (Anthos reader 2001, Anthos, Siegburg, Germany).

A positive reaction was defined by two criteria: the absorption of serum had to be at least twice as high as a negative control and at least 0.10 higher than the reaction of the serum itself on the RSV uncoated control plate.

Statistics

The data were evaluated using the SAS statistical package. Differences between the RSV group and the non-infected group were tested for significance with the chi-square coefficient and Fisher's two-tailed exact test, when appropriate. The relative risk

Fig. 1. Percentages of specific sensitizations against aeroallergens depending on RSV antibody status at 12 months



(RR) and 95% confidence intervals (CI) for sensitization were calculated. Logistic regression technique was used to control for confounders. A p value < 0.05 was accepted as significant.

Results

RSV antibodies

Of the cord sera tested 99% were seropositive for specific RSV-IgG antibodies reflecting the mothers' immunity. At 12 months, 44.7%, and after 24 months, 64.2% were seropositive for specific IgG. The level of IgG antibodies in the cord blood did not predict the antibody status at one year. RSV-IgM was found in 12% of the children at one, and in 23.4% at two years of age. Antibody prevalence did not differ between infants at risk for atopy and the controls.

Seasonal effects – One-year-old children born in the first three months of the year ($n=151$) had a higher prevalence for RSV-IgG (54.1%) than those born during the following quarters ($n=420$, prevalence 52.3 to 44.4%, $p=0.006$ for trend). This pattern was not seen after the second year of life. At this later age seropositivity for IgG ranged between 56–70%, and for IgM between 15–27% according to the quarter.

Siblings – RSV IgG seropositivity was seen in 53.7% of infants with older siblings ($n=258$), compared with 39.4% for firstborn children ($n=289$, $p=0.001$). After two years, seropositivity was seen

in 75% of children with older siblings, compared with 55.9% of those with none ($p=0.001$).

Exposure to tobacco smoke – At one year of age, RSV IgG-antibody positivity was found to have a dose-dependent association with passive smoking: 108/284 (38%) of the infants living in non-smoking families were seropositive for RSV, whereas 87/174 (50%) were seropositive in families with one, and 52/93 (56%) in families with two or more smokers ($p=0.002$ for the 3×2 table).

Clinical data

The incidence of wheezing was recorded as one of the asthma-like symptoms (9). During the first month of life, no such symptoms occurred in our study population. The more convincing the history (obvious symptoms) the higher was the prevalence of RSV seropositivity at one year ($p=0.001$). Probable (57 [10.1%] at 12 months and 29 [11.7%] at 24 months) or obvious (44 [7.9%] at 12 months and 18 [7.6%] at 24 months) asthmatic symptoms were reported in infants at atopic risk and controls in equal numbers.

Atopic sensitization

The total IgE at 12 and 24 months of age did not differ between groups with (median: 6.35 kU/l at 1 year; 15.1 kU/l at 2 years of age) or without (median: 6.1 kU/l; 20.6 kU/l) RSV antibodies. The num-

Table 1. Sensitization and RSV antibodies: the incidence of sensitization against at least one aeroallergen depending on the RSV antibody prevalence, and the relative risk (RR) with the 95% confidence interval (CI) for sensitization at one year.

RSV-Antibodies	IgM positive	IgM negative	RR (95% CI)
IgG positive	3/56 (5.3%)	5/210 (2.3%)	2.2 (0.56-9.1), $p=0.37$
IgG negative	2/16 (12.5%)	10/315 (3.1%)	3.9 (0.94-16.4), $p=0.1$
all	5/72 (6.9%)	15/515 (2.9%)	2.4 (0.91-6.5), $p=0.08$

ber of infants sensitized to at least one of the eight allergens tested was 89 (14.8%) after the first year, and 68 (25.8) after the second year of life. Twenty children (2.8%) with at least one sensitization against inhalant allergens were found at 12 months, and 28 (10.5%) at 24 months. The respective figures for nutritional allergens were 80 (13.1%) and 53 (20%). Children of the risk group became sensitized easier than controls (Sensitization against at least one allergen: 22.3% versus 10.2% at 12 months [$p<0.001$], 31% versus 18.4% [$p=0.002$] at 24 months).

At 12 months, infants with a sensitization against any inhalant allergen showed no higher prevalence for RSV IgG-antibodies (40% versus 44.7%; *n.s.*). Table 1 gives the incidence of sensitization according to the RSV antibody status. Specific sensitization against dermatophagoides pteronyssinus ($n=3$) was only seen in RSV IgG seropositive children. Infants sensitized against birch pollen showed a higher prevalence for RSV IgM (60% were seropositive, compared with an 11.2% seropositivity in the unsensitized group; $p=0.014$). The same applied to those sensitized against grass pollen (40% versus 12%; $p=0.015$). Table 2 gives the number of specific sensitizations, and the relative risk of RSV seropositivity. Figure 1 shows the percentages of specific sensitization related to RSV seropositivity.

RSV infections during the second year of life, diagnosed by seroconversion to RSV-IgG or RSV-IgM seropositivity at two years, were not predictive for sensitization against any of the eight allergens at 24 months.

In a first logistic regression model, recognized factors for atopic sensitization such as sex, breast-feeding, exposure to smoking, RSV infections, season of birth, and membership of the high risk group for atopy were tested for their effect on inhalant sensitization in general. Only members of the high risk group had a higher rate of sensitization at one year ($p=0.0016$). RSV-IgM seropositivity at one year had a small effect on sensitization ($p=0.093$). Exposure to smoking and the season of birth had no effect on sensitization.

In a second model, the same factors for specific sensitization against pollen at one year were evaluated. Being seropositive for RSV IgM or RSV IgG

Table 2. Relative risk for specific inhalant sensitization at one year of age depending on RSV-antibody status. P-values from Fisher's exact test 2-tailed. *) RR not calculated due to a zero sensitization in the RSV IgG negative group

Sensitization	RR of RSV antibody positivity (C.I.)	
	IgG	IgM
birch ($n=5$)	1.8 (0.30-11) $p=0.66$	11.3 (1.8-68) $p=0.014$
grass ($n=9$)	0.62 (0.15-2.5) $p=0.73$	6.2 (1.6-23) $p=0.015$
cat ($n=8$)	0.62 (0.17-3.1) $p=0.73$	2.4 (0.48-12.3) $p=0.25$
mite ($n=3$)	*)	3.5 (0.3-40) $p=0.32$

was associated with grass pollen sensitization (IgG $p=0.048$, IgM $p=0.0006$). Sensitization against birch pollen was only associated with seropositivity for RSV-IgM ($p=0.009$). Season of birth, exposure to smoking, sex or membership of the high risk group did not influence the prevalence of sensitization against grass or birch pollen.

Allergic manifestations

RSV-antibody status was not associated with any other atopic manifestation such as eczema, gastrointestinal reactions or rhinoconjunctivitis.

Discussion

The aim of this study was to evaluate prospectively the role of RSV infection in early sensitization against inhalant allergens and the early manifestations of atopic disease.

In order to obtain reliable results from such an analysis, the method of detecting RSV infections must be clearly understood. In comparison with some earlier authors (11) we were not in a position to examine every participant whenever she or he caught a cold and to take a sample and test it for RSV antigen. Since taking convalescent sera is neither an adequate nor a standard diagnostic procedure (12), we could not - for ethical reasons - obtain a sufficient number of sera from infants with a proven RSV infection during their first year of life to make a classical sensitivity/specificity analysis of the ELISA used. However, using this test we could reproduce the recognized positive risk factors for early RSV infection. For those who are seropositive at one year, these include passive smoking (13-15), having older siblings and the season of birth (11). Furthermore, seropositivity on the first birthday correlated almost exactly with the incidence of wheezing, which is the predominant feature of an RSV infection in infancy, irrespective of the atopic family history (16-19). This makes us confident that the measurement of the RSV antibody reliably reflects the occurrence of the RSV infection in our sample population.

With regard to early specific sensitization, one first has to ensure that the total IgE at one year of age is not affected by the RSV antibody status. This is plausible, since total IgE at one year is best predicted by the cord blood IgE (20). On the other hand, RSV is one of the viruses known for its low interferon induction (21), and it is thus very unlikely to influence negatively the production of total IgE.

When considering the specific sensitizations, it is remarkable how house dust mite sensitization occurred in RSV infected infants only during the first year, even if the number of cases was too small to be statistically significant. The higher prevalence of RSV-IgM in the group of birch and grass sensitized infants also indicates the temporary adjuvant role of the RSV infection in sensitization by aeroallergens during the first year of life. The association of RSV IgM seropositivity (which means a recent infection) with sensitization to seasonal inhalant allergens may be confirmation of an early report by Frick (6) on older children. It is also in accordance with results obtained from animal models (1, 2). A synopsis of the latter publications allows us to regard the underlying mechanism as a facilitation of sensitization by anatomical damage. This view is further supported by a case control study on children hospitalized for RSV bronchiolitis, who became prone to asthma as well as to inhalant sensitization up to the age of three years (22).

According to our investigation, the effect of RSV modulation on aeroallergen sensitization of non hospitalized infants does not persist into the second year of life. From this age onward, the risk of sensitization resulting from an atopic family history clearly overrides the risk due to RSV infection (7, 19, 23). For this reason, we and other authors (11, 24) have been unable to detect any modifying effect of RSV infection, when looking for the atopic manifestations during infancy and early childhood which depend for the most part on sensitization to ingested allergens. Only in the prospective study on infants hospitalized for bronchiolitis food-IgE in three-year-olds was found more often in patients than in controls. However, this study included highly selected RSV patients, which is confirmed by the fact, that among them allergic sensitization was influenced more by bronchiolitis than by an atopic heredity.

The observation that multiple respiratory infections may offer protection against sensitization (25) is not contradictory to our own observations. In fact, RSV is only a poor inducer of interferon in comparison with other viruses, such as rhinovirus influenzae and the viruses of influenza and parainfluenza, which also produce more clinical signs in older children (21). These latter viral infections are readily recalled by the parents in a retrospective study, and may prevent sensitization in accordance with the

concept of an interferon driven upregulation of Th1-like helper cells.

Taken as a whole, this prospective study fills in the gap in our knowledge of what RSV infection signifies for aeroallergen sensitization in non-hospitalized children. During the first year of life it slightly promotes aeroallergen sensitization. This does not depend upon the atopic status. There is no demonstrable effect on atopic manifestations in the first two years of life, since these depend principally upon the atopic disposition of the child.

Acknowledgement

The study was supported by a grant from the German Federal Ministry of Science and Technology, Bonn, Germany.

References

1. RIEDEL F. Influence of adjuvant factors on development of allergy. Evidence from animal experiments. *Pediatr Allergy Immunol* 1991; 2: 1-5.
2. LEIBOVITZ E, FREIHORST J, PIEDRA PA, OGRA PL. Modulation of systemic and mucosal immune response to inhaled ragweed antigen in experimentally induced infection with Respiratory Syncytial Virus. Implication in virally induced allergy. *Int Arch Allergy Immun.* 1988; 86: 112-6.
3. GERSHWIN LJ, HIMES SR, DUNGWORTH DL, GIRI SN, FRIEBERTSHAUSER KE, CAMACHO M. Effect of Bovine Respiratory Syncytial Virus infection on hypersensitivity to inhaled *Micropolyspora faeni*. *Int Arch Allergy Immunol* 1994; 104: 79-91.
4. SIGURS N, BJARNASON R, SIGURBERGSSON F. Asthma and IgE antibodies after respiratory syncytial virus bronchiolitis. *J Allergy Clin Immunol* 1994; Abstr. 198.
5. WELLIVER RC, SUN M, RIJNALDO D, OGRA PL. Predictive value of respiratory syncytial virus-specific IgE response for recurrent wheezing following bronchiolitis. *J Pediatr* 1986; 109: 776-80.
6. FRICK OL, GERMAN DF, MILLS J. Development of allergy in children. I. Association with virus infections. *J Allergy Clin Immunol* 1979; 63: 228-41.
7. STARK JM, BUSSE WW. respiratory virus infection and airway hyperreactivity in children. *Pediatr Allergy Immunol* 1991; 2: 95-110.
8. MUTIUS V E, FRITZSCH C, WEILAND SK, ROLL G, MAGNUSSEN H. Prevalence of asthma and allergic disorders among children in united Germany: a descriptive comparison. *BMJ* 1992; 305 (6866): 1395-9.
9. BERGMANN RL, BERGMANN KE, LAU S, LUCK W, DANNE-MANN A, BAUER CP, DORSCH W, FORSTER J, et al. Atopic diseases in infancy. The German multicenter atopy study (MAS-90). *Pediatr Allergy Immunol* 1994; Suppl 5: 19-25.
10. STRECKERT HJ, WERCHAU H. Epitopes at the proteolytic cleavage sites of HIV-1-gp120 and RSV-F protein share a sequence homology: comparative studies with virus-induced and antipeptide antibodies. *Intervirology* 1992; 34: 30-7.
11. HOLBERG CJ, WRIGHT AL, MARTINEZ FD, RAY CG, TAUSSIG LM, LEBOWITZ MD, et al. Risk factors for respiratory syncytial virus-associated lower respiratory illnesses in the first year of life. *Am J Epidemiol* 1991; 133: 1135-51.
12. American Academy of Pediatrics. Respiratory syncytial virus, In: Peter G, ed. 1994 Red Book: Report of the Com-

- mittee on Infectious Diseases. 23rd ed. Elk Grove Village IL: American Academy of Pediatrics: 1994: 396–8.
13. Jin C, ROSSIGNOL AM. Effects of passive smoking on respiratory illness from birth to age eighteen months, in Shanghai, People's Republic of China. – *J Pediatr* 1993; 123: 553–8.
14. WRIGHT AL, HOLBERG C, MARTINEZ FD, TAUSSIG LM. Relationship of parental smoking to wheezing and non wheezing lower respiratory tract illness in infancy. *J Pediatr* 1991; 118: 207–14.
15. MAGNUSSEN CG. Maternal smoking influences cord serum IgE and IgD levels and increases the risk for subsequent infant allergy. *J Allergy Clin Immunol* 1986; 78: 898–904.
16. SIMS DG, GARDNER PS, WEIGHTMAN D, TURNER MW, SOOTHILL JF. Atopy does not predispose to RSV bronchiolitis or postbronchiolitic wheezing. *BMJ* 1981; 282: 2086–8.
17. WEISS ST, TAGER IB, MUNOZ A, SPEIZER FE. The relationship of respiratory infections in early childhood to the occurrence of increased levels of bronchial responsiveness and atopy. *Am Rev Respir Dis* 1985; 131: 573–8.
18. VAN ASPEREN PP, MUKHI A. The role of atopy in the natural history of wheeze and bronchial-hyper-responsiveness in childhood. *Pediatr Allergy Immunol* 1994; 5: 178–83.
19. COGSWELL JJ, HALLIDAY DF, ALEXANDER JR. Respiratory infections in the first year of life in children at risk of developing atopy. *BMJ* 1982; 284: 1011–3.
20. BERGMANN RL, SCHULZ J, GÜNTHER S, DUDENHAUSEN JW, BERGMANN KE, BAUER CP, DORSCH W et al. Determinants of cord-blood IgE concentrations in 6401 German neonates. *Allergy* 1995; 50: 65–71.
21. HALL C, DOUGLAS R, SIMONS R, GEIMAN M. Interferon production in children with respiratory syncytial, influenza, parainfluenza virus infections. *J Pediatr* 1978; 93: 28–32.
22. SIGURS N, BJARNASON R, SIGURBERGSSON F, KJELLMAN B, BJÖRKSTEN B. Asthma and Immunglobulin E antibodies after respiratory syncytial virus bronchiolitis: A prospective cohort study with matched controls. *Pediatrics* 1995; 95: 500–5.
23. BACKER V, ULRIK CS, BACH MN, GLIKMANN G, MORDHORST CH. Relationship between viral antibodies and bronchial hyper-responsiveness in unselected children and adolescents. *Allergy* 1993; 48: 240–7.
24. HALONEN M, STERN D, TAUSSIG LM, WRIGHT A, RAY CG, MARTINEZ FD. The predictive relationship between serum IgE levels at birth and subsequent indices of lower respiratory illnesses and eczema in infants. *Am Rev Respir Dis* 1992; 146: 866–70.
25. V MUTIUS E, MARTINEZ FD, FRITZSCH C, NICOLAI T, REITMEIR P, THIEMANN HH. Skin test reactivity and number of siblings. *BMJ* 1994; 308: 692–5.