

Serum concentrations of interferon- γ and intercellular adhesion molecule-1 eight years after an early respiratory syncytial virus infection

H. Juntti*, J. Kokkonen*, T. Dunder*, M. Renko*, R. Karttunen† and M. Uhari*

Departments of *Paediatrics and †Medical Microbiology, University of Oulu, Finland

Summary

Background Respiratory syncytial virus (RSV) infection may influence the development of recurrent wheezing and atopy, but the mechanisms are unclear.

Objective The purpose was to evaluate serum concentrations of soluble intercellular adhesion molecule-1 (sICAM-1), CD14, IgE, IL-5 and IFN- γ in children 6–10 years after an RSV infection and their correlation with subsequent asthma and atopy.

Methods Fifty-one subjects admitted to hospital for RSV infection during the first year of life and controls matched for birth date and sex underwent clinical examinations including lung function, skin prick and blood tests.

Results The RSV subjects had significantly higher serum concentrations of IFN- γ and sICAM-1 than the controls (for IFN- γ 224.9 pg/mL (standard deviation (SD) 271.3) vs. 187.1 pg/mL (372.9), difference 37.8 pg/mL, 95% confidence interval (CI) –90.3 to 166.0, $P = 0.05$; for sICAM-1 170.2 ng/mL (SD 63) vs. 147.8 ng/mL (SD 57), difference 22.4 ng/mL, 95% CI –1.4 to 46.1, $P = 0.04$). The RSV subjects with asthma had significantly higher concentrations of IFN- γ than the controls with asthma, and the RSV subjects with wheezing during the previous 12 months had significantly higher concentrations of both IFN- γ and sICAM-1 than the controls with wheezing.

Conclusions Children hospitalized for RSV infection in infancy still differ in IFN- γ and sICAM-1 production 6–10 years after the infection. The data suggest that the pathomechanism of asthma and wheezing after an early RSV infection may be different from that of children without an early RSV infection.

Keywords atopy, IFN- γ , respiratory syncytial virus, skin prick test, soluble ICAM-1

Submitted 12 December 2003; revised 18 June 2004; accepted 22 July 2004

Introduction

Respiratory syncytial virus (RSV) infection is often the first significant respiratory infection among children, nearly all of whom contract it by the age of 2 [1]. It has been thought to influence the development of recurrent wheezing and atopy, but the mechanisms for this are not well known [2–4]. It is possible that an underlying allergic predisposition or some other genetically regulated mechanism may affect the type of immune response induced during the infection and its severity [5, 6].

IFN- γ , produced by mononuclear cells, is an important component of the early cellular response to RSV, although the levels of T-helper type 2 (Th2)-type cytokines have also been found to be up-regulated in many studies [7–9]. IFN- γ may also be important in the pathogenesis of virus-induced wheezing, possibly through the induction of leukotriene release [10]. Th2-type cytokines, especially IL-5 and concomitant eosinophils, have been thought to play a major role in asthma and airway hyper-responsiveness (AHR), but there

are also studies in which IFN- γ levels have been elevated in bronchoalveolar lavage (BAL) samples from asthmatic children [11–13]. In a mouse model of allergic asthma, IFN- γ antibodies abolished the development of airway hyper-reactivity in ovalbumin (OVA)-challenged mice [14].

Soluble intercellular adhesion molecule-1 (sICAM-1) is important for eosinophil and neutrophil adhesion and may be pivotal for the pathogenesis of AHR and asthma [15]. IFN- γ induces ICAM-1 production, which can also be induced directly by RSV [16]. Up-regulation of the ICAM-1 gene in the airway epithelial cells during an acute paramyxoviral infection has been shown in a mouse model to be associated with inflammation and hyper-reactivity [17]. Elevated levels of sICAM-1 in serum might even be a marker of persistent airway inflammation, as seen in children with asthma even when symptom free [18].

CD14 has been shown to act as a receptor for RSV [19]. There is a promoter polymorphism in the gene CD14, which associates with atopy and IgE production and the concentration of soluble CD14 (sCD14) in the serum [20].

We evaluated sICAM-1, sCD14, IgE and cytokine IL-5 and IFN- γ concentrations in a follow-up study of children admitted to hospital because of an RSV infection during

Correspondence: Hanna Juntti, Department of Paediatrics, University of Oulu, PO Box 5000, FIN-90014, University of Oulu, Finland.

E-mail: hanna.juntti@oulu.fi

their first year of life and controls matched for sex and date of birth. The results were also correlated with the occurrence of airway hyper-reactivity, atopic diseases and sensitization in the subjects and controls. Our hypothesis was that children experiencing a severe RSV infection in early infancy may either have been selected by the virus because of differences in their immunological parameters or else the infection may have changed their immune response permanently so that they still differ from the controls years after the infection in terms of their immune response and/or hypersensitivity. These differences may also affect the development of subsequent atopy and asthma.

Materials and methods

We enrolled to the original material all the 117 children who had been admitted to Oulu University Hospital between 1991 and 1994 because of an RSV infection during their first year of life and in whom RSV had been confirmed either with an ELISA antigen or by the latex agglutination test. Children born prematurely (< 37 gestational weeks), those with a need for respiratory assistance > 6 h during the neonatal period and those with a concomitant disease such as congenital heart disease, chromosome abnormality, or severe neurological disease were excluded ($n = 30$). The children were invited to participate in the current survey 6–10 years after the infection, and 51 (30 females, mean age 7.8 years (range 6.9–10.3)) agreed to participate. A detailed study profile and an account of the means of selecting the controls, matched for sex and date of birth, are described elsewhere [21]. The follow-up visit included a clinical examination, lung function tests and skin prick tests (SPTs) with 15 common allergens, and also blood tests to determine the serum concentrations of IgE, IL-5, IFN- γ , sCD14 and sICAM-1.

The definitions of symptoms and diagnoses of atopic diseases were based on the questions used in the International Study of Asthma and Allergic disease in Childhood (ISAAC) [22]. In cases where asthma was suspected because of recurrent wheezing or chronic cough, the child was examined further by an independent paediatric allergologist. Those diagnosed as having asthma were classified accordingly. A family history of atopic diseases was also recorded.

The protocols for the skin prick testing and lung function tests are described elsewhere [21].

Cytokines and CD14

IgE concentrations were determined by the laboratory's routine methods. Serum samples stored at -20°C were measured for IFN- γ , IL-5, sCD14 and sICAM-1 levels with commercial EIA tests (IFN- γ ; R&D, sCD14 and sICAM-1; HyCult Biotechnology, Uden, The Netherlands). The lower detection limits were 120 pg/mL for IFN- γ , 1 $\mu\text{g/mL}$ for sCD14 and 63 ng/mL for sICAM-1.

Statistical analysis

The data were analysed with SPSS for Windows version 11.0. χ^2 -test, Fisher's exact test and the Mann-Whitney U -test were used to test the significances of differences between the groups. The Spearman rank correlation was used to determine the correlations between concentrations. The difference between two correlation coefficients was tested as described by Snedecor and Cochran [23]. Logistic regression analysis was used to analyse the influence of RSV infection and a family history of asthma and allergy on the occurrence of asthma and atopic diseases. Odds ratios (ORs) and 95% confidence intervals (CIs) were calculated. We decided not to use paired tests, since the RSV subjects and controls were matched only for their date of birth and sex.

Ethical considerations

The study protocol was found acceptable by the Ethical Committee of Oulu University Hospital. The parents gave their written informed consent.

Results

There was a small but significant difference between the RSV subjects and controls in their age at the time of examination, caused by the duration of the selection process (Table 1). There were no significant differences in the other demographic and background variables.

Table 1. Demographic characteristics of the RSV subjects and controls

	RSV subjects ($n = 51$)*	Controls ($n = 51$)*	Difference between the groups (95% CI)†
Age at examination (years): mean (range)	7.8 (6.9–10.3)	8.0 (7.1–10.3)	– 0.20 (– 0.58 to 0.18)‡
No. of siblings: mean (range)	4.04 (0–16)	3.00 (0–14)	1.04 (– 0.31 to 2.38)‡
No. of acute otitis media episodes: median (range)	7.0 (0–20)	4.0 (0–20)	
Duration of breastfeeding; months: mean (range)	6.45 (0–19)	7.98 (2.5–24)	– 1.53 (– 3.35 to 0.29)‡
Mother smoking during pregnancy: no. (%)	5 (10)	10 (20)	– 10% (– 24 to 4)‡
Smoking in the household during the previous year: no. (%)	23 (45)	17 (33)	12% (– 7 to 3)‡
Pets at home at the time of the examination: no. (%)	18 (35)	27 (53)	– 18% (– 36 to 2)‡
Type of day care at 1–2 years of age: no. (%), home	44 (88)	40 (78)	10% (– 3 to – 23%)‡

CI, confidence interval.

* $n = 48$ among the respiratory syncytial virus (RSV) subjects and 50 among the controls for the number of acute otitis media episodes, $n = 49$ for duration of breastfeeding among the RSV subjects and $n = 50$ in the RSV subjects for type of day care at 1–2 years because of missing information.

†By Student's t -test.

‡Difference in proportions.

The occurrence of asthma was slightly higher among the RSV subjects than the controls, but the difference was not significant (Table 2). No significant differences in the results of the lung function tests or in the use of medication were found between the RSV subjects and controls with either asthma or wheezing during the previous 12 months (data not shown), nor were there any differences in the occurrence of other atopic diseases. Eight percent of the RSV subjects had at least one positive SPT, compared with 43% of the controls (difference -35%, 95% CI -50% to -19%, $P < 0.0001$). No significant differences were found in the family history of asthma and/or atopy.

The mean serum concentration of IFN- γ was significantly higher among the RSV subjects (Table 3) and this cytokine was more often detectable in these (16 out of 51) than in the controls (eight out of 51). The mean sICAM-1 concentration was also significantly higher in the RSV subjects, but no differences in sCD14 or IgE concentrations were found between the groups, and IL-5 was not detectable in any of the samples. There was a significant correlation between the serum concentrations of IFN- γ and sICAM-1 among both the RSV subjects and the controls ($r = 0.680$, $P < 0.001$ and $r = 0.284$, $P = 0.044$, respectively), but the concentration of sCD14 showed no correlation with either IFN- γ or sICAM-1.

We found no significant differences in IgE concentrations between the RSV subjects and controls with asthma (difference -155 kU/L, 95% CI -405 to 96 kU/L), wheezing during the previous 12 months (difference -249 kU/L, 95% CI -573 to 76 kU/L), allergic rhinitis (AR) (difference -235 kU/L, 95% CI -881 to 411 kU/L), atopic dermatitis (difference -20 kU/L, 95% CI -181 to 140 kU/L) or at least one positive SPT (difference -159 kU/L, 95% CI -533 to 215 kU/L).

IFN- γ was detectable in five RSV subjects with asthma ($n = 15$) and six with wheezing during the previous 12 months

($n = 13$) but in none of the controls with asthma ($n = 11$) or wheezing ($n = 13$) (Table 4). The mean IFN- γ concentration in the RSV subjects with or without asthma (174.7 vs. 245.9, $P = 0.98$) or with or without wheezing during the previous 12 months (335.1 vs. 187.3, $P = 0.15$) showed no significant difference. IFN- γ was undetectable in all the samples from RSV subjects ($n = 3$) and controls ($n = 8$) with AR and in all four RSV subjects and 19 out of the 22 controls with at least one positive SPT. No significant differences in the concentrations of IFN- γ were found between the RSV subjects and controls with atopic dermatitis (difference 186.8 pg/mL, 95% CI -23.4 to 396.9 pg/mL).

No significant differences in sCD14 concentrations were found between the RSV subjects and controls with either asthma (difference -0.08 μ g/mL, 95% CI -0.4 to 0.3 μ g/mL), wheezing during the previous 12 months (difference -0.2 μ g/mL, 95% CI -0.5 to 0.2 μ g/mL), AR (difference 0.2 μ g/mL, 95% CI -0.7 to 1.0 μ g/mL), atopic dermatitis (difference -0.04 μ g/mL, 95% CI -0.3 to 0.3 μ g/mL) or at least one positive SPT (difference -0.2 μ g/mL, 95% CI -0.3 to 0.7 μ g/mL).

sICAM-1 concentrations were significantly higher in the RSV subjects with wheezing during the previous 12 months than in the corresponding controls (mean 199.2 ng/mL, SD 96.7 vs. 127.9 ng/mL, SD 47.7, $P = 0.026$, difference 71.3 ng/mL, 95% CI 9.6 to 133.0 ng/mL), the difference being most pronounced among the children with wheezing and negative SPTs (difference 95 ng/mL, 95% CI 16.5 to 174.2 ng/mL, $P = 0.007$). The mean ICAM-1 concentration in the RSV subjects with or without asthma (161.1 vs. 173.9 ng/mL, $P = 0.93$) and with or without wheezing during the previous 12 months (199.2 vs. 160.2, $P = 0.19$) did not differ significantly and neither did sICAM-1 concentrations between the RSV subjects and controls with either a diagnosis of asthma (difference 23.8 ng/mL, 95% CI -8.5 to 56.0 ng/mL), AR (difference -0.6 ng/mL, 95% CI -67.3 to 66.2 ng/mL) or at least one positive SPT (difference -18.3 ng/mL, 95% CI -61.6 to 25.1 ng/mL). The RSV subjects with atopic dermatitis had significantly higher sICAM-1 concentrations than the corresponding controls (mean 179.1 ng/mL, SD 61.2 vs. 136.7 ng/mL, SD 28.8, $P = 0.044$, difference 42.3 ng/mL, 95% CI 9.3 to 75.4 ng/mL).

In the logistic regression analysis, RSV infection was found to be associated with a decreased risk of at least one positive SPT (OR 0.1, 95% CI 0.04 to 0.4, $P < 0.001$), and together with a positive family history of asthma and/or atopy with an increased risk of asthma (OR 2.8, 95% CI 1.1 to 7.0, $P = 0.03$). A family history of asthma and/or allergy alone was associated only with an increased risk of atopic dermatitis (OR 6.5, 95% CI 1.4 to 29.6, $P = 0.016$).

Table 2. Asthma and atopy among the subjects and controls; symptoms and diagnoses, no. (%)

	RSV subjects ($n = 51$)	Controls ($n = 51$)	Odds ratio	95% CI	P -value*
Asthma	15 (29)	11 (22)	1.52	0.62 to 3.72	0.36
Wheezing during the previous 12 months	13 (26)	13 (26)	1.00	0.41 to 1.44	1.00
Allergic rhinitis	3 (6)	8 (16)	0.34	0.08 to 1.35	0.20
Atopic dermatitis	15 (29)	18 (35)	0.76	0.33 to 1.76	0.53
At least one positive skin prick test	4 (8)	22 (43)	0.11	0.04 to 0.36	<0.001

RSV, respiratory syncytial virus; CI, confidence interval.

*By χ^2 -test (Fisher's exact test, when $n < 5$).

Table 3. Concentrations of S-IgE, IFN- γ , soluble CD14 and ICAM-1 in the serum of the RSV subjects and controls

	RSV subjects ($n = 51$)	Controls ($n = 51$)	Difference	95% CI	P *
S-IgE (kU/L): mean (SD)	119.2 (176.7)	244.1 (343.2)	-124.9	-232.1 to -17.6	0.17
IFN- γ (pg/mL): mean (SD)	224.9 (271.3)	187.1 (372.9)	37.9	-90.3 to 166.0	0.05
Soluble CD14 (μ g/mL): mean (SD)	1.84 (0.42)	1.83 (0.40)	0.01	-0.1 to 0.2	0.8
Soluble ICAM-1 (ng/mL): mean (SD)	170.0 (63)	148.0 (57)	22.3	-1.4 to 46.1	0.04

RSV, respiratory syncytial virus; ICAM-1, intercellular adhesion molecule-1; CI, confidence interval, SD, standard deviation.

*By Mann-Whitney U -test.

Table 4. Concentrations of IFN- γ (pg/mL) in serum of the RSV subjects and controls with asthma or atopy

	RSV subjects* mean (SD)	Controls* mean (SD)	P†
Asthma	174.7 (101.1)	120 (0)	0.04
Wheezing during the previous 12 months	335.1 (445.7)	120 (0)	0.007
Atopic dermatitis	320.0 (435.9)	133.2 (44.2)	0.08
At least one positive skin prick test	120 (0)	131.4 (48.9)	0.4

SD, standard deviation.

* $n = 15$ for respiratory syncytial virus (RSV) subjects and $n = 11$ for controls with asthma, $n = 13$ for RSV subjects and controls with wheezing during the previous 12 months, $n = 15$ for RSV subjects and $n = 18$ for controls with atopic dermatitis, $n = 4$ for RSV subjects and $n = 22$ for controls with at least one positive skin prick test.

†By Mann-Whitney *U*-test.

Discussion

Our main finding was that children hospitalized for RSV infection early in infancy still had significantly higher serum concentrations of a Th1-type cytokine, IFN- γ , and of sICAM-1 than controls matched for birth date and sex 6–10 years after the infection. There was a close correlation between the concentrations of IFN- γ and sICAM-1 in the RSV subjects, while the correlation was weaker, although still significant, in the controls. Our results indicate that RSV infection may be capable of identifying children genetically pre-determined to produce high amounts of IFN- γ and sICAM-1 and thus having a tendency to wheeze during viral respiratory infections. With our set-up, however, we cannot exclude the possibility that an early RSV infection may induce long-term immunomodulatory changes in the lymphoregulatory system, as seen from the rises in IFN- γ and sICAM-1. We found no measurable IL-5 in the serum of RSV subjects, but as we measured the cytokine concentrations only in the serum and did not perform any up-regulation studies on the airway mucosa, there remains a possibility that some local up-regulation of IL-5 could have been induced [15].

Both increased and decreased IFN- γ production has been reported during an acute RSV infection [8, 9, 14, 24–28], as detected in either nasopharyngeal, BAL or serum samples or blood mononuclear cells. In some studies the youngest and often prematurely born children had the most severe infection, with a lower concentration of IFN- γ than the healthy controls or subjects with upper respiratory infections [8]. By contrast, children with a milder disease, although still severe enough to lead to wheezing and hospitalization, had elevated levels of IFN- γ [25, 27]. Our RSV subjects had had a moderate infection, because there was only one who had required mechanical ventilation, yet they were still IFN- γ producers. On the other hand, the asthmatic controls had different amounts of IFN- γ from those of asthmatic RSV subjects, suggesting that IFN- γ may not have been as important for wheezing or asthma among the RSV subjects as among the controls. The fact that IFN- γ induces ICAM-1 production most probably explains the significant correlation between them [17].

There are few studies on the long-term effects of the immune response after RSV infection. Pala et al. [29] studied children admitted at less than 12 months of age for an infection at a mean age of 7.5 years and found a higher frequency of IL-4-secreting T cells in the peripheral blood in response to stimulation with RSV than in controls, but their methods differed considerably from ours, as we measured only spontaneous secretion of cytokines and not stimulated secretion. The Th2-type cytokine IL-5 was undetectable in all our samples.

IFN- γ was undetectable in all our controls with asthma, but was detectable in five out of 15 RSV subjects with asthma. The fact that none of these five had any positive SPTs suggests a non-atopic mechanism. Increased levels of sICAM-1 and IFN- γ have been found in asthmatic children and children with recurrent wheezing, with the highest levels of sICAM-1 in cases with a severe disease [13]. Our RSV subjects with wheezing during the previous 12 months had significantly higher concentrations of both IFN- γ and sICAM-1 than the controls with wheezing, with the highest concentrations of sICAM-1 occurring in the RSV subjects with both wheezing and negative SPTs. These findings may suggest differences in the mechanisms by which asthma and wheezing are mediated in children with and without an early RSV infection. No differences were found between the asthmatic RSV subjects and controls in their use of medication or in the results of lung function tests.

Our finding of more positive SPTs in the controls than in the RSV subjects may be related to the fact that the controls were selected at random from the general population and thus represent an unselected series of children. Similar sensitization rates have been reported in other Finnish studies [30, 31]. A similar negative association between RSV infection and SPT positivity has been reported on one occasion previously, while in most other cases equal or higher-than-expected incidences have been reported [32, 33]. We found no difference in the concentrations of sICAM-1 and IFN- γ between the RSV subjects and controls with positive SPTs. The RSV subjects with atopic dermatitis had significantly higher sICAM-1 concentrations than the controls. A close correlation has been found elsewhere between sICAM-1 and the severity of atopic dermatitis, but we did not evaluate the severity aspect here [34].

In conclusion, the association between RSV infection, recurrent wheezing and the development of atopy remains unclear. Although we suggest that RSV identifies children with a tendency to wheeze, it may induce an immune deviation affecting subsequent immune responses in these children. Our RSV subjects had less atopy as measured by positive skin prick tests and a higher concentration of the Th1-type cytokine IFN- γ .

Acknowledgements

This study was supported in part by a grant from the Foundation for Paediatric Research, Finland; the Finnish Cultural Foundation, Regional Fund for Northern Ostrobothnia; the Alma and K. A. Snellman Foundation, Oulu, Finland; the Research Foundation of Orion Corporation and the Allergy Research Foundation.

References

- Ruuskanen O, Ogra PL. Respiratory syncytial virus. *Curr Probl Pediatr* 1993; 23:50–79.
- Noble V, Murray M, Webb MS, Alexander J, Swarbrick AS, Milner AD. Respiratory status and allergy nine to 10 years after acute bronchiolitis. *Arch Dis Child* 1997; 76:315–9.
- Sigurs N, Bjarnason R, Sigurbergsson F, Kjellman B. Respiratory syncytial virus bronchiolitis in infancy is an important risk factor for asthma and allergy at age 7. *Am J Respir Crit Care Med* 2000; 161:1501–7.
- Schwarze J, Hamelmann E, Bradley KL, Takeda K, Gelfand EW. Respiratory syncytial virus infection results in airway hyperresponsiveness and enhanced airway sensitization to allergen. *J Clin Invest* 1997; 100:226–33.
- Trefny P, Stricker T, Baerlocher C, Sennhauser FH. Family history of atopy and clinical course of RSV infection in ambulatory and hospitalized infants. *Pediatr Pulmonol* 2000; 30:302–6.
- Lofgren J, Ramet M, Renko M, Marttila R, Hallman M. Association between surfactant protein A gene locus and severe respiratory syncytial virus infection in infants. *J Infect Dis* 2002; 185:283–9.
- Hussell T, Openshaw PJ. Intracellular IFN- γ expression in natural killer cells precedes lung CD8⁺ T cell recruitment during respiratory syncytial virus infection. *J Gen Virol* 1998; 79: 2593–601.
- Aberle JH, Aberle SW, Dworzak MN et al. Reduced interferon- γ expression in peripheral blood mononuclear cells of infants with severe respiratory syncytial virus disease. *Am J Respir Crit Care Med* 1999; 160:1263–8.
- Tripp RA, Moore D, Barskey A et al. Peripheral blood mononuclear cells from infants hospitalized because of respiratory syncytial virus infection express T helper-1 and T helper-2 cytokines and CC chemokine messenger RNA. *J Infect Dis* 2002; 185:1388–94.
- van Schaik SM, Tristram DA, Nagpal IS, Hintz KM, Welliver RC, Welliver RC. Increased production of IFN- γ and cysteinyl leukotrienes in virus-induced wheezing. *J Allergy Clin Immunol* 1999; 103:630–6.
- van Schaik SM, Welliver RC, Kimpen JL. Novel pathways in the pathogenesis of respiratory syncytial virus disease. *Pediatr Pulmonol* 2000; 30:131–8.
- Hessel EM, van Oosterhout AJ, Van AI et al. Development of airway hyperresponsiveness is dependent on interferon- γ and independent of eosinophil infiltration. *Am J Respir Cell Mol Biol* 1997; 16:325–34.
- Marguet C, Dean TP, Warner JO. Soluble intercellular adhesion molecule-1 (sICAM-1) and interferon- γ in bronchoalveolar lavage fluid from children with airway diseases. *Am J Respir Crit Care Med* 2000; 162:1016–22.
- Walter MJ, Morton JD, Kajiwarra N, Agapov E, Holtzman MJ. Viral induction of a chronic asthma phenotype and genetic segregation from the acute response. *J Clin Invest* 2002; 110: 165–75.
- Schwarze J, Cieslewicz G, Hamelmann E et al. IL-5 and eosinophils are essential for the development of airway hyperresponsiveness following acute respiratory syncytial virus infection. *J Immunol* 1999; 162:2997–3004.
- Wegner CD, Gundel RH, Reilly P, Haynes N, Letts LG, Rothlein R. Intercellular adhesion molecule-1 (ICAM-1) in the pathogenesis of asthma. *Science* 1990; 247:456–9.
- Stark JM, Godding V, Sedgwick JB, Busse WW. Respiratory syncytial virus infection enhances neutrophil and eosinophil adhesion to cultured respiratory epithelial cells. Roles of CD18 and intercellular adhesion molecule-1. *J Immunol* 1996; 156:4774–82.
- Tang RB, Chen SJ, Soong WJ, Chung RL. Circulating adhesion molecules in sera of asthmatic children. *Pediatr Pulmonol* 2002; 33:249–54.
- Kurt-Jones EA, Popova L, Kwinn L et al. Pattern recognition receptors TLR4 and CD14 mediate response to respiratory syncytial virus. *Nat Immunol* 2000; 1:398–401.
- Baldini M, Lohman IC, Halonen M, Erickson RP, Holt PG, Martinez FD. A polymorphism* in the 5' flanking region of the CD14 gene is associated with circulating soluble CD14 levels and with total serum immunoglobulin E. *Am J Respir Cell Mol Biol* 1999; 20:976–83.
- Juntti H, Kokkonen J, Dunder T, Renko M, Niinimäki A, Uhari M. Association of an early respiratory syncytial virus infection and atopic allergy. *Allergy* 2003; 58:878–84.
- Asher MI, Keil U, Anderson HR et al. International Study of Asthma and Allergies in Childhood (ISAAC): rationale and methods. *Eur Respir J* 1995; 8:483–91.
- Snedecor GW, Cochran WG. Correlation. *Statistical methods* 1980; 186–7.
- Mobbs KJ, Smyth RL, O'Hea U, Ashby D, Ritson P, Hart CA. Cytokines in severe respiratory syncytial virus bronchiolitis. *Pediatr Pulmonol* 2002; 33:449–52.
- Bont L, Heijnen CJ, Kavelaars A et al. Local interferon- γ levels during respiratory syncytial virus lower respiratory tract infection are associated with disease severity. *J Infect Dis* 2001; 184:355–8.
- Chen ZM, Mao JH, Du LZ, Tang YM. Association of cytokine responses with disease severity in infants with respiratory syncytial virus infection. *Acta Paediatr* 2002; 91:914–22.
- Garofalo RP, Patti J, Hintz KA, Hill V, Ogra PL, Welliver RC. Macrophage inflammatory protein-1 α (not T helper type 2 cytokines) is associated with severe forms of respiratory syncytial virus bronchiolitis. *J Infect Dis* 2001; 184:393–9.
- Brandenburg AH, Kleinjan A, van Het LB et al. Type 1-like immune response is found in children with respiratory syncytial virus infection regardless of clinical severity. *J Med Virol* 2000; 62:267–77.
- Pala P, Bjarnason R, Sigurbergsson F, Metcalfe C, Sigurs N, Openshaw PJ. Enhanced IL-4 responses in children with a history of respiratory syncytial virus bronchiolitis in infancy. *Eur Respir J* 2002; 20:376–82.
- Remes ST, Korppi M. Asthma and atopy in schoolchildren in a defined population. *Acta Paediatr* 1996; 85:965–70.
- Kosunen TU, Hook-Nikanne J, Salomaa A, Sarna S, Aromaa A, Haahtela T. Increase of allergen-specific immunoglobulin E antibodies from 1973 to 1994 in a Finnish population and a possible relationship to *Helicobacter pylori* infections. *Clin Exp Allergy* 2002; 32:373–8.
- Gentile DA, Doyle WJ, Zeevi A et al. Cytokine gene polymorphisms moderate illness severity in infants with respiratory syncytial virus infection. *Hum Immunol* 2003; 64:338–44.
- Peebles RS Jr, Hashimoto K, Collins RD et al. Immune interaction between respiratory syncytial virus infection and allergen sensitization critically depends on timing of challenges. *J Infect Dis* 2001; 184:1374–9.
- Culley FJ, Pollott J, Openshaw PJ. Age at first viral infection determines the pattern of T cell-mediated disease during reinfection in adulthood. *J Exp Med* 2002; 196:1381–6.