Allergy 2005: 60: 226–232

ALLERGY
DOI: 10.1111/j.1398-9995.2005.00684.x

Original article

Age- and infection-related maturation of the nasal immune response in 0–2-year-old children

Background: The hygiene hypothesis suggests that exposure to micro-organisms influences development of the immune system in children.
Methods: In this study, we examined nasal immune responses in the first 2 years of life in relation to age of children and the number of viral infections they have experienced. Nasal brushes were taken during rhinovirus- ($n = 20$) or respiratory syncytial virus (RSV)-induced ($n = 7$) upper respiratory tract infections (URTI), and of controls ($n = 40$).
Results: The number of macrophages were higher during URTI and increased with age. The number of T lymphocytes increased with age in controls and were higher during URTI at all ages. We found an age-related decrease in the number of interleukin (IL)-4- and IL-10-positive cells in controls, while the number of IL-12-positive cells remained unchanged. Changes in T lymphocyte and IL-4 cell number were stronger related to the age of the child than to the number of respiratory infections, while the opposite was true for macrophages.
Conclusions: In infants, we found an infection- and age-related increase respectively for nasal macrophages and T lymphocytes during URTI. Furthermore, the number of IL-4- and IL-10-positive cells decreased with age. Whether this maturation reflects a natural age-related maturation, the degree of exposure to respiratory infections, or possibly both, could not be resolved and needs further study.

I. J. van Benten[1], C. M. van Drunen[1], L. P. Koopman[2], B. C. van Middelkoop[1], W. C. J. Hop[3], A. D. M. E. Osterhaus[4], H. J. Neijens[2], W. J. Fokkens[1]

Departments of [1]Otorhinolaryngology, [2]Paediatrics, and [3]Epidemiology and Biostatistics, [4]Institute for Virology, Erasmus Medical Center, Rotterdam, The Netherlands

Present address: Cornelis M. van Drunen, Wytske J. Fokkens, Department of Otorhinolaryngology, Academic Medical Center, Amsterdam, The Netherlands

Key words: infant; interleukin-4; interleukin-10; interleukin-12; maturation; respiratory infection; rhinovirus; respiratory syncytial virus.

Dr C. M. van Drunen
Department of Otorhinolaryngology
room L3-351 Academic Medical Center
PO box 22700, 1100 DE Amsterdam
The Netherlands

Accepted for publication 7 June 2004

The immune system in infants is not fully developed. This is reflected by the high susceptibility of children to respiratory infections during the first few years of life (1). In general, host immune responses to viral pathogens are mediated by T-helper (Th)1-type cytokines (2). As the production of Th1 cytokines can be inhibited by cytokines secreted by Th2 lymphocytes (3), an adequate balance of Th2 and Th1 cytokines is essential for the efficient eradication of pathogens.

The high risk of infections in infants may be a consequence of an immature cellular host immune response. *In vitro* experiments with peripheral and cord blood cells have shown that cells from infants respond less well to infection-related stimuli, than those from adults, with a lower production of both Th1-like cytokines [interleukin (IL)-2, IL-12, interferon (IFN)γ] and Th2-like cytokines (IL-5) (4–6). Furthermore, the production of Th2 cytokines is favoured over Th1 cytokines, in infants (7). This may result from a preferential Th2 milieu that prevails during pregnancy (8).

The development of the child's immune system towards adult-like Th1 cytokine production progresses over time. Several studies on peripheral blood T lymphocytes and mononuclear cells showed that Th1-like cytokine production [IL-2, IL-12, IFNγ, tumour necrosis factor (TNF)α] increased with age after stimulating cells *in vitro* with polyclonal or infection-related stimuli (9, 10). Furthermore, it has been suggested that maturation of the immune system in children who will become allergic during later life, shows a different pattern than that of healthy nonallergic children (11).

Hardly anything is known about factors affecting or regulating the maturation of the immune system. The hygiene hypothesis (12) suggests that changes in lifestyle may have contributed to a reduction in the exposure to micro-organisms of individual children. As successive Th1 responses may steer the child's immune system towards a mature Th1 response in adulthood, the reduced exposure could result in a diminished maturation of their immune system. The Th2-skewed immune

response in infants may therefore persevere and predispose a child to the later development of a Th2-mediated allergic disease.

No data are available about whether postulated changes in the developing immune system of young children are also reflected in the nasal mucosa. In our study, we therefore examined whether the magnitude of nasal macrophage and T-lymphocyte responses, as well as Th1-driving (IL-12), Th2-driving (IL-4) and regulatory cytokine responses (IL-10), during upper respiratory tract infections (URTI) in 0–2-year-old children, were affected by age and/or the history of respiratory tract infections.

Materials and methods

Participants and data collection

In all, 24 infants (age 2–22 months, eight boys) participating in the VIGALL birth cohort study (13) were examined during 27 episodes of URTI in the first 2 years of life either caused by rhinovirus ($n = 20$) or by respiratory syncytial virus (RSV) ($n = 7$). URTI was defined as a runny nose and at least one of the symptoms such as fever, malaise, sleeping difficulties, or loss of appetite. This case–control study was confined to children in whom a single type of virus was detected during URTI. Sixteen children had a positive family history of atopy (FHA; one or both parents had allergic disease) and 11 children had a negative FHA (no allergic disease in the parents). Parents with self-reported asthma, hay fever, house dust mite allergy, or pet allergy were considered to be allergic. This was established with a validated screening questionnaire (14).

A control group comprised 36 other children (age 6–26 months, 18 boys) from the same birth cohort study (matched for FHA, age, and gender) were completely free of any symptoms (no runny nose, fever, malaise, cough, wheeze, sleeping difficulties, or loss of appetite) in the 2 weeks spanning the visit. The children had no virus infection during sampling, and had not suffered from atopic dermatitis before the age of 2 years (determined using the UK Working Party's Diagnostic Criteria for Atopic Dermatitis) (15).

During each visit, a physical examination was performed and nasal brush samples were taken. A medical history was taken for general illness symptoms (fever, general malaise, loss of appetite), upper respiratory tract disease (runny nose, sore throat), and lower respiratory tract disease (wheezing, cough, dyspnoea) in the preceding follow-up period. General symptoms of illness such as runny nose, cough, fever, and symptoms of allergic disease such as skin rash and wheezing were scored by the parents on weekly symptom cards during the first 2 years of life. The Medical Ethics Committee of the Erasmus Medical Centre Rotterdam approved the study design and all parents gave informed consent.

Cumulative incidence of respiratory infections

The number of respiratory infections experienced by children from birth to each visit, and the total duration of those infections, were calculated on the basis of runny nose symptoms registered on weekly symptom cards. If more than 30% of the weekly symptom cards filled in prior to sampling were incomplete, data were not analysed for that child. When 70–100% of the weekly symptom cards were scored correctly (53 of 67 visits), the number of respiratory infections and their total duration from birth up to visit were extrapolated from the available data.

Viral diagnostics

Cells were harvested from the nose with a cytobrush (Medscand Medical, Sweden) and collected in 7 ml of RPMI 1640 medium (Life Technologies, Breda, the Netherlands). After centrifugation, the supernatant was used to detect influenzavirus, parainfluenzavirus, RSV, adenovirus, cytomegalovirus (CMV), enterovirus and echovirus on cultured HEp-2 cells with immunofluorescence. Additionally, nasal brush cells from the pellet were stained directly with fluorescent-labelled antiviral antibodies to detect RSV, influenzavirus, parainfluenzavirus and adenovirus (16). Finally, rhinovirus and coronavirus were detected by the isolation of viral RNA from nasal brush supernatant using reverse transcriptase-polymerase chain reaction (RT-PCR), followed by virus-specific hybridization (17).

Immunohistochemical detection of macrophages and T lymphocytes

The immunohistochemical staining procedure was as described elsewhere (18) to detect macrophages (αCD68, 4.6 μg/ml, clone EBM11; DAKO, Glostrup, Denmark) and T lymphocytes (αCD3, 4.2 μg/ml, clone T3-4B5; DAKO) in nasal brush samples with an isotypic antibody as negative control. The number of positive cells with red cytoplasmic and/or cell membrane staining were counted per 1000 nasal brush cells and stated as percentages of positive cells.

Immunohistochemical detection of IL-4-, IL-10-, and IL-12-positive cells

The super-sensitive alkaline phosphatase staining method (19) was used to detect cytokine positive cells in nasal brush samples. Cytospin preparations of nasal brush cells were incubated with mouse anti-human monoclonal antibodies directed against IL-4 (12 μg/ml, clone 1-41-1; Novartis, Basel, Switzerland), IL-10 (10 μg/ml, clone IC25-471; Instruchemie, Delfzijl, The Netherlands) or IL-12p70 (5 μg/ml, clone 24945.11; R&D Systems, Abingdon, UK) or an isotypic control antibody. The number of positive cells with red cytoplasmic staining were counted per 1000 nasal brush cells and were stated as percentages of positive cells.

Statistical analysis

The relationship between the number of effector cells and cytokine-positive cells on the one hand and age and FHA on the other hand were investigated with regression analysis for repeated measurements (using the 'proc mixed' module from SAS 6.12 for windows; SAS, Cary, NC, USA). In order to obtain approximate linear relations in these analyses, the outcomes were transformed logarithmically. The same method was used to evaluate the effects of number of respiratory infection episodes. Differences were considered statistically significant when the P-value was ≤ 0.05.

Results

Age-related macrophage and T-lymphocyte responses during URTI

In control children, the number of macrophages was low (range 0–2%) and this did not depend on the age of the child. In the group of children with URTI, we observed a marked increase of macrophages in the nose at every age ($P < 0.001$) (Fig. 1A). Moreover, the number of macrophages attracted to the nose during URTI

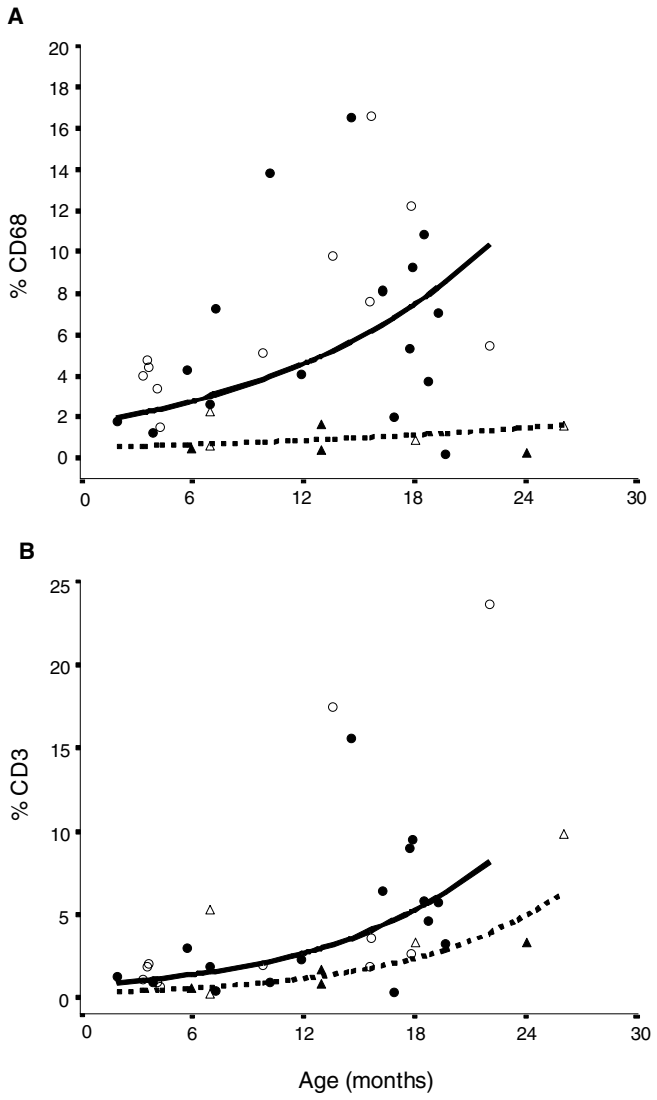

Figure 1. The age-related increase in percentages of (A) nasal macrophages (CD68-positive) and (B) T lymphocytes (CD3 positive) in children during URTI (circles, solid line; 27 samples in 24 children) and in controls (triangles, dashed line; 11 samples in 11 children) with a positive (closed symbols) or a negative FHA (open symbols). Curves correspond to linear regression lines after logarithmic transformation of the vertical axis.

increased 2.7-fold per 12 months of age ($P = 0.02$; Table 1). A URTI episode in 6-month-old children resulted in an increase in the median percentage of macrophages from 1% at baseline to 3% during infection, whereas this percentage increased to 10% in children aged 22 months. We did not find any difference in number of macrophages between RSV and rhinovirus infections at any time point, nor were the number of macrophages dependent on the FHA of the child.

The number of T lymphocytes in nasal brushes of these children also revealed age-related changes (Fig. 1B). In contrast to the macrophage response, a comparable

Table 1. Relation between immunological parameters and the age of the child or the number of respiratory infections experienced

	Age	*P*-value	Adjusted *P*-value*	Respiratory infections	*P*-value	Adjusted *P*-value†
URTI						
Macrophages	2.7 (2.2–3.4)	0.02	NS	1.7 (1.1–2.5)	0.02	0.05
T lymphocytes	3.8 (1.9–7.6)	0.01	0.05	1.5 (1.1–2.2)	0.04	NS
Baseline						
IL-4	0.6 (0.5–0.9)	0.01	0.09	0.8 (0.7–1.0)	0.08	NS
IL-10	0.8 (0.6–1.0)	0.09	NS	0.9 (0.8–1.1)	NS	NS
IL-12	1.1 (0.6–2.0)	NS	NS	1.1 (0.8–1.5)	NS	NS

Data given are the factors (95% confidence interval) at which the levels increase per 12 months of age or per doubling of the number of respiratory infections. *P*-values showing a statistically significant relation ($P \leq 0.05$) and *P*-values showing a trend ($P \leq 0.10$) are represented (NS, not significant).

* *P*-value for the relation with age adjusted for number of respiratory infections.

† *P*-value for relation with number of respiratory infections adjusted for age.

increase in the number of T lymphocytes with the advancing age of the child was observed in controls ($P = 0.01$) and during URTI ($P = 0.01$). The median percentage of T lymphocytes of controls increased from 0.5% at 6 months of age to 4% at 22 months. During URTI, an influx of T lymphocytes into the nose was observed at all ages ($P = 0.03$), which was comparable between rhinovirus and RSV infections. At the age of 6 months, the median number of T lymphocytes increased from 0.5% at baseline to 1% in children with URTI, while in 22-month-old children this increased from 4 to 8%. This represents a 3.8-fold increase per 12 months of age during URTI (Table 1). Moreover, here the changes in T lymphocytes did not depend on the FHA of the child.

Age-related cytokine responses

Figure 2A shows a significant age-dependent decrease in the number of Th2-driving cytokine IL-4-positive cells ($P = 0.01$), which decreased from a median number of 20% at 6 months to 10% at 24 months [factor 0.6 (40%) per 12 months of age] (Table 1). For the regulatory cytokine IL-10, a trend was observed towards an age-related decrease in the number of cells ($P = 0.09$; Table 1). The median number of IL-10-positive cells were reduced from 41% at 6 months to 29% at 24 months of age (Fig. 2B). The median number of the Th1-driving IL-12 cytokine-positive cells remained unchanged (median 6%) with age (Fig. 2C). No significant differences were found in nasal cytokine responses between children with a positive or negative FHA.

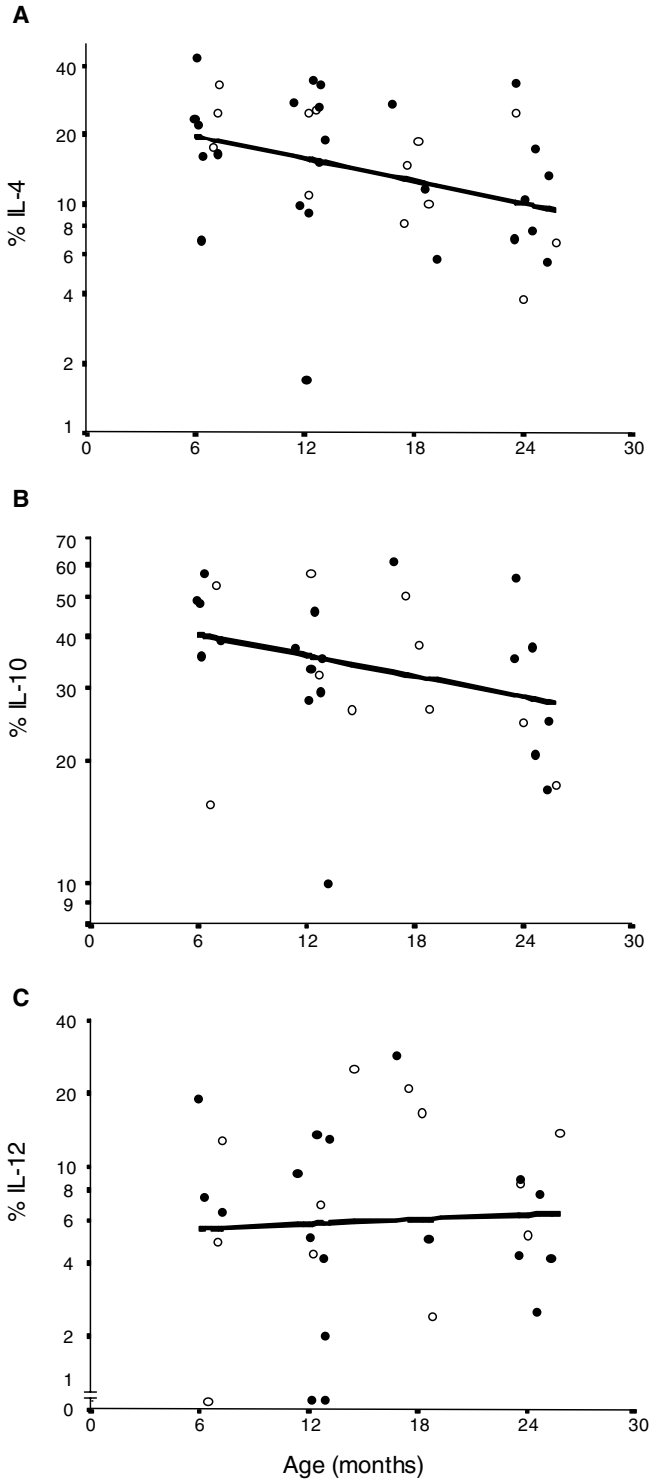

Figure 2. Age-related dependence of (A) nasal IL-4-positive cells (38 samples in 33 children), (B) IL-10 positive cells (29 samples in 25 children), and (C) IL-12-positive cells (30 samples in 26 children) in control children with a positive (closed symbols) or a negative FHA (open symbols).

Age-related or infection-related immune maturation in children?

The cumulative incidence of respiratory infections increased from approximately two episodes at 6 months to approximately three at 12 months, approximately five at 18 months and approximately eight episodes at 24 months of age ($P < 0.001$) and did not depend on the FHA. As the cumulative incidence of respiratory infections was positively related to age, we would expect the nasal immune responses to be also related to the number of respiratory infections. A positive relation was indeed found between number of respiratory infections and the number of macrophages or T lymphocytes ($P = 0.02$ and 0.04, respectively; $n = 20$). The number of macrophages increased 1.7-fold and T lymphocytes 1.5-fold per doubling of the number of respiratory infections (Table 1). Additionally there was a trend towards a decrease in IL-4-positive cells ($P = 0.08$, $n = 32$) (Table 1), while no relation was found between IL-12 ($n = 30$) or IL-10 ($n = 26$) and the number of respiratory infections.

To discriminate between age-related or infection-related maturation, we performed a regression analysis for repeated measurements that included both variables. However, because of the high correlation between the age of the child and the number of respiratory infections, the effect of both variables on the IL-10 response proved difficult to separate. The strongest predictor for the T lymphocyte response during URTI and the IL-4 response in children during baseline turned out to be the age of the child. The age of the child remained related to both responses, even after adjusting for the number of respiratory infections ($P = 0.05$ for T lymphocytes, $P = 0.09$ for IL-4; Table 1). This is supported by the observation that, when the relation between the number of respiratory infections and the IL-4 and T-lymphocyte responses was adjusted for age, this relation was not statistically significant ($P = 0.77$ and 0.85, respectively). However, the strongest predictor for the macrophage response during URTI turned out to be the number of respiratory infections, as the relation remained statistically significant, even after adjusting for the age of the child ($P = 0.05$; Table 1).

Discussion

The hygiene hypothesis suggests that exposure to microorganisms contributes to the development of the immune system in children. This study is the first to show an age-related nasal immune maturation in 0–2-year-old children, with number of Th2-driving (IL-4) and regulatory (IL-10) cytokine-positive cells decreasing with age. Interestingly, the percentages of nasal macrophages and T lymphocytes attracted to the nose during URTI were low until the age of 6 months, but increased rapidly thereafter with the age of the child. In 2-year-old children, the

number of macrophages and T lymphocytes from control children and from the group of children with URTI were comparable with those in adult patients with a common cold, as reported in our previous study (20). This indicates that recruitment of inflammatory cells to the nose is in full potential at the age of 2 years. An assessment of whether the age-related increase in the number of T lymphocytes in nasal brush samples is due to an increase of a particular type of T lymphocytes was not possible, but memory T lymphocytes are likely candidates. While children show high percentages of naïve T lymphocytes (approximately 82%) and only few memory T lymphocytes (approximately 16%) in peripheral blood, adult T lymphocytes show the opposite phenotype (approximately 48% naïve, 49% memory) (21).

A change in the natural balance between Th1 and Th2 cytokine production with the age of the child from Th2-skewed at birth to Th1-skewed in adulthood has been suggested (7). This could explain the age-related increase in effector cell responses observed during URTI. Indeed, in our study, an age-related decrease in Th2-driving (IL-4) cytokine-positive cells was observed in the noses of children. It is difficult to relate our *in vivo* data to known *in vitro* data obtained from cord blood and peripheral blood samples. Prescott and colleagues found an age-related decrease in the expression of Th2 cytokine IL-4 mRNA in allergen-stimulated cord blood and peripheral-blood mononuclear cells in 0–18-month-old children (22). However, others found an age-related increase in Th2 cytokine responses during the first year of life when peripheral blood cells were activated polyclonally or with allergen (9, 23).

In the present study also, the number of nasal IL-10-positive cells decreased with age. These high IL-10 responses in the nose of infants are in line with studies showing a high expression of IL-10 by peripheral blood mononuclear cells of infants compared with adults (24). The role of IL-10 in immune maturation is complex. IL-10 can inhibit the production of Th1-driving cytokine IL-12 by dendritic cells (25), and therefore a decrease in the number of nasal IL-10-positive cells with age would allow an age-related increase in nasal IL-12 responses. This in turn would stimulate Th1 cytokine production, as IL-12 is a key factor for Th1 differentiation (26). Recently it has become clear that IL-10 can inhibit many more cytokines (TNFα, IL-1α, IL-1β, IL-2, IL-5, IL-6, IL-18) and therefore IL-10 is nowadays regarded as an important regulatory cytokine (27). In this quality high expression of IL-10 in infancy may induce tolerance in early life and may regulate a balanced Th1 and Th2 maturation. This regulatory role of IL-10 in immune maturation has already been suggested by van den Biggelaar et al. (28), who found that high production of IL-10 during helminth infection was inversely related to the development of Th2-mediated allergic disease in children.

The reduction in the number of IL-10- and IL-4-positive cells is not paralleled by an increase in number of Th1 driving IL-12-positive cells. Although this does not preclude an increase in protein production. Lower production of IL-12 protein by LPS-stimulated neonatal DCs has been observed by comparison with adult DCs (29). Recently, Upham et al. (30) showed that protein production of IL-12p70 increased gradually from infancy to adulthood after stimulation of peripheral and cord-blood cells. Unfortunately, our approach did not allow us to determine whether levels of IL-12 protein expression rather than cell number are upregulated.

Children with a positive or negative FHA did not differ in terms of nasal cytokine responses (IL-4, IL-10, IL-12). This concurs with findings from in vitro studies where polyclonally stimulated cord blood T lymphocytes from both groups of children did not differ in the production of Th1 and Th2 cytokines (31). However, the absence of a difference between children with positive or negative FHA could also stem from the relative small number of children included in the study. This small sample size also did not allow us the study the same children for 2 years both during and outside an URTI episode. Differences in cytokine production have been observed between children that do and do not develop allergic disease. Van der Velden et al. (11) showed that protein levels of Th2 cytokines (IL-4) produced by polyclonally stimulated blood lymphocytes increased from birth to 12 months of age in children with a high genetic risk for allergy who developed allergic disease at 2 years of age, whereas cytokine levels did not change in children who remained healthy. However, as none of our children had developed atopic dermatitis at the age of 2 years and a proper diagnosis of asthma and allergic rhinitis cannot be made in these young children, we were not able to verify these observations in our study. A follow-up study on the development of allergic disease at the age of 6 years will help us address this question.

The hygiene hypothesis suggests that repeated respiratory infections influence immune maturation in children towards stronger Th1 responses and limit Th2 responses, resulting in a diminished chance of developing Th2-mediated allergic disease (12). This is partly supported by the present study as macrophage responses during URTI were more strongly related to the increasing number of respiratory tract infections than to the age of the child. However, our data on the development of nasal IL-4 and T-lymphocyte responses show that these responses were more strongly related to the age of the child. At present, we are not able to distinguish between a strict age-related or infection-related immune maturation and it may well be that both factors are important for a correct maturation. We have necessarily restricted ourselves to viral infections in our study and not evaluated possible effects of bacterial infections or colon colonisation. Given our results, a larger study seems called for that encompasses these factors.

No differences were observed in children with a high or a low genetic risk of developing allergic disease. Neither

the incidence of respiratory infections nor the nasal macrophage and T lymphocyte response upon URTI differed, indicating that both groups of children are equally susceptible to respiratory infections. This is in agreement with studies showing that a parental history of allergic disease is a risk factor for lower respiratory tract infections, but not for URTI (32). The design of our study also allowed an analysis of potential differences on the effect of upper *vs* lower respiratory tract infections on immune maturation. This would be interesting, as we have previously shown a strong nasal IL-18 response in RSV-induced lower respiratory tract infection in children, where such a response is absent during a RSV-induced URTI (33).

Acknowledgments

We would like to thank all parents and children for their participation in the VIGALL study and Novartis for kindly providing the αIL-4 antibody. This study is supported by the Dutch Asthma Foundation (Nr 93.96.1), the Netherlands Organisation for Health Science (Nr 940-35-025), and the Foundation 'Vereniging Trustfonds Erasmus Universiteit Rotterdam' (Nr 97030) in the Netherlands.

References

1. Denny FW Jr. The clinical impact of human respiratory virus infections. Am J Respir Crit Care Med 1995;**152**:S4–S12.
2. Moore BB, Moore TA, Toews GB. Role of T- and B-lymphocytes in pulmonary host defences. Eur Respir J 2001;**18**:846–856.
3. Parronchi P, De Carli M, Manetti R, Simonelli C, Sampognaro S, Piccinni MP et al. IL-4 and IFN (alpha and gamma) exert opposite regulatory effects on the development of cytolytic potential by Th1 or Th2 human T cell clones. J Immunol 1992;**149**:2977–2983.
4. Kruse A, Neustock P, Reuter M, Kirchner H. T-cell surface molecule expression and interferon-gamma production in human cord blood. J Interferon Res 1993;**13**:221–225.
5. Lee SM, Suen Y, Chang L, Bruner V, Qian J, Indes J et al. Decreased interleukin-12 (IL-12) from activated cord versus adult peripheral blood mononuclear cells and upregulation of interferon- gamma, natural killer, and lymphokine-activated killer activity by IL- 12 in cord blood mononuclear cells. Blood 1996;**88**:945–954.
6. Pit DS, Polderman AM, Schulz-Key H, Soboslay PT. Prenatal immune priming with helminth infections: parasite-specific cellular reactivity and Th1 and Th2 cytokine responses in neonates. Allergy 2000;**55**:732–739.
7. Ribeiro-do-Couto LM, Boeije LC, Kroon JS, Hooibrink B, Breur-Vriesendorp BS, Aarden LA et al. High IL-13 production by human neonatal T cells: neonate immune system regulator? Eur J Immunol 2001;**31**:3394–3402.
8. Wegmann TG, Lin H, Guilbert L, Mosmann TR. Bidirectional cytokine interactions in the maternal-fetal relationship: is successful pregnancy a TH2 phenomenon? Immunol Today 1993;**14**:353–356.
9. Buck RH, Cordle CT, Thomas DJ, Winship TR, Schaller JP, Dugle JE. Longitudinal study of intracellular T cell cytokine production in infants compared to adults. Clin Exp Immunol 2002;**128**:490–497.
10. Elsasser-Beile U, Dursunoglu B, Gallati H, Monting JS, von Kleist S. Comparison of cytokine production in blood cell cultures of healthy children and adults. Pediatr Allergy Immunol 1995;**6**:170–174.
11. van der Velden VH, Laan MP, Baert MR, de WaalMalefyt R, Neijens HJ, Savelkoul HF. Selective development of a strong Th2 cytokine profile in high-risk children who develop atopy: risk factors and regulatory role of IFN- gamma, IL-4 and IL-10. Clin Exp Allergy 2001;**31**:997–1006.
12. Strachan DP. Family size, infection and atopy: the first decade of the "hygiene hypothesis". Thorax 2000;**55**(Suppl. 1):S2–S10.
13. Koopman LP, Van Benten IJ, Dewaal L, Fokkens WJ, Osterhaus ADME, Neijens HJ. Virus mediated allergy (Vigall)-study: study design and clinical results 1 year follow-up. Am J Resp Crit Care Med 2000;**161**:A347.
14. Lakwijk N, Van Strien RT, Doekes G, Brunekreef B, Gerritsen J. Validation of a screening questionnaire for atopy with serum IgE tests in a population of pregnant Dutch women. Clin Exp Allergy 1998;**28**:454–458.
15. Williams HC, Forsdyke H, Boodoo G, Hay RJ, Burney PG. A protocol for recording the sign of flexural dermatitis in children. Br J Dermatol 1995;**133**: 941–949.
16. Rothbarth PH, Habova JJ, Masurel N. Rapid diagnosis of infections caused by respiratory syncytial virus. Infection 1988;**16**:252.
17. Pitkaranta A, Arruda E, Malmberg H, Hayden FG. Detection of rhinovirus in sinus brushings of patients with acute community-acquired sinusitis by reverse transcription-PCR. J Clin Microbiol 1997;**35**:1791–1793.
18. Godthelp T, Holm AF, Fokkens WJ, Doornenbal P, Mulder PG, Hoefsmit EC et al. Dynamics of nasal eosinophils in response to a nonnatural allergen challenge in patients with allergic rhinitis and control subjects: a biopsy and brush study. J Allergy Clin Immunol 1996;**97**:800–811.
19. Braunstahl GJ, Overbeek SE, Fokkens WJ, Kleinjan A, McEuen AR, Walls AF et al. Segmental bronchoprovocation in allergic rhinitis patients affects mast cell and basophil numbers in nasal and bronchial mucosa. Am J Resp Crit Care Med 2001;**164**:858–865.
20. van Benten IJ, KleinJan A, Neijens HJ, Osterhaus AD, Fokkens WJ. Prolonged nasal eosinophilia in allergic patients after common cold. Allergy 2001;**56**:949–956.
21. Beck R, Lam-Po-Tang PR. Comparison of cord blood and adult blood lymphocyte normal ranges: a possible explanation for decreased severity of graft versus host disease after cord blood transplantation. Immunol Cell Biol 1994;**72**:440–444.
22. Prescott SL, Macaubas C, Smallacombe T, Holt BJ, Sly PD, Holt PG. Development of allergen-specific T-cell memory in atopic and normal children. Lancet 1999;**353**:196–200.
23. Vigano A, Esposito S, Arienti D, Zagliani A, Massironi E, Principi N et al. Differential development of type 1 and type 2 cytokines and beta- chemokines in the ontogeny of healthy newborns. Biol Neonate 1999;**75**:1–8.

24. Rainsford E, Reen DJ. Interleukin 10, produced in abundance by human newborn T cells, may be the regulator of increased tolerance associated with cord blood stem cell transplantation. Br J Haematol 2002;**116**:702–709.
25. De Smedt T, Van Mechelen M, De Becker G, Urbain J, Leo O, Moser M. Effect of interleukin-10 on dendritic cell maturation and function. Eur J Immunol 1997;**27**:1229–1235.
26. Macatonia SE, Hosken NA, Litton M, Vieira P, Hsieh CS, Culpepper JA et al. Dendritic cells produce IL-12 and direct the development of Th1 cells from naive CD4+ T cells. J Immunol 1995;**154**:5071–5079.
27. Moore KW, de Waal Malefyt R, Coffman RL, O'Garra A. Interleukin-10 and the interleukin-10 receptor. Annu Rev Immunol 2001;**19**:683–765.
28. van den Biggelaar AH, van Ree R, Rodrigues LC, Lell B, Deelder AM, Kremsner PG et al. Decreased atopy in children infected with *Schistosoma haematobium*: a role for parasite-induced interleukin-10. Lancet 2000;**356**:1723–1727.
29. Langrish CL, Buddle JC, Thrasher AJ, Goldblatt D. Neonatal dendritic cells are intrinsically biased against Th-1 immune responses. Clin Exp Immunol 2002;**128**:118–123.
30. Upham JW, Lee PT, Holt BJ, Heaton T, Prescott SL, Sharp MJ et al. Development of interleukin-12-producing capacity throughout childhood. Infect Immun 2002;**70**:6583–6588.
31. Hagendorens MM, Van Bever HP, Schuerwegh AJ, De Clerck LS, Bridts CH, Stevens WJ. Determination of T-cell subpopulations and intracellular cytokine production (interleukin-2, interleukin-4, and interferon-gamma) by cord blood T-lymphocytes of neonates from atopic and non-atopic parents. Pediatr Allergy Immunol 2000;**11**:12–19.
32. Koopman LP, Smit HA, Heijnen ML, Wijga A, van Strien RT, Kerkhof M et al. Respiratory infections in infants: interaction of parental allergy, child care, and siblings-the piama study. Pediatrics 2001;**108**:943–948.
33. van Benten IJ, van Drunen CM, Koopman LP, KleinJan A, Hop WCJ, van Middelkoop BC et al. RSV-induced bronchiolitis but not upper respiratory tract infection is accompanied by an increased nasal IL-18 response. J Med Virol 2003;**71**:290–297.