

RSV-Induced Bronchiolitis But Not Upper Respiratory Tract Infection Is Accompanied by an Increased Nasal IL-18 Response

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The aim of this study was to investigate potential differences in the local nasal immune response between bronchiolitis and upper respiratory tract infection induced by respiratory syncytial virus (RSV). Nasal brush samples were obtained from 14 infants with RSV bronchiolitis and from 8 infants with RSV upper respiratory tract infection. The samples were taken during infection (acute phase) and 2–4 weeks later (convalescent phase). Cytospin preparations were stained immunohistochemically for T cells, macrophages, and eosinophils. Staining also took place for intercellular adhesion molecule-1 (ICAM-1), T-helper 1 (Th1)-like (interleukin-12 [IL-12], interferon- γ [IFN- γ]), Th2-like (IL-4, IL-10), and proinflammatory cytokines (IL-6, IL-8, IL-18). During both RSV-induced bronchiolitis and upper respiratory tract infection, cellular inflammation was observed. This was characterised by an increase in the numbers of nasal macrophages, which tended to be higher in bronchiolitis than in upper respiratory tract infection. Numbers of T lymphocytes and ICAM-1 positive cells increased during both bronchiolitis and upper respiratory tract infection. There were no differences between numbers in the groups. Interestingly, a distinct nasal proinflammatory cytokine response was observed in RSV-induced bronchiolitis. This is characterised by an increase in the number of IL-18 positive cells. This increase is specific for bronchiolitis, as a similar increase could not be detected in RSV-induced upper respiratory tract infection. Numbers of IL-6 and IL-12 positive cells were higher in both bronchiolitis and upper respiratory tract infection, and there were no differences between the groups. By contrast, the number of IL-8, IFN- γ , IL-4, and IL-10-positive cells remained constant. In conclusion, clear differences were found in nasal immune responses of children

with RSV-induced upper respiratory tract infection or bronchiolitis. The induction of a strong IL-18 response was typical for bronchiolitis, as this could not be observed in RSV-induced upper respiratory tract infection, and could explain the eosinophilia that is observed frequently during bronchiolitis. **J. Med. Virol.** 71:290–297, 2003. © 2003 Wiley-Liss, Inc.

KEY WORDS: cytokines; infant; bronchiolitis; RSV

INTRODUCTION

Respiratory syncytial virus (RSV) infection in young children can either be restricted to the upper respiratory tract, leading to a simple cold, or include the lower airways and result, for example, in bronchiolitis [Fisher et al., 1997]. Although RSV-induced bronchiolitis is nearly always preceded by symptoms of upper respiratory tract infection, it is not clear why an RSV infection becomes more severe in some cases. An interesting question is whether there could be differences between

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the nasal immune responses of RSV-induced bronchiolitis and RSV-induced upper respiratory tract infection.

The immunological effects of RSV infections are quite diverse and seem to encompass T-helper 1 and 2 (Th1, Th2), and proinflammatory responses. Increased levels of typical Th2 markers, such as eosinophil cationic protein (ECP) and RSV-specific IgE, have been found in nasal lavage samples during bronchiolitis in infants [Welliver and Duffy, 1993; Garofalo et al., 1994]. In line with the increased levels of ECP, higher peripheral blood eosinophil counts have also been observed [Garofalo et al., 1994; Ehlenfield et al., 2000]. In addition, stimulated peripheral blood mononuclear cells (PBMCs) revealed enhanced Th2 cytokine responses in infants during RSV bronchiolitis compared with controls [Roman et al., 1997]. Proinflammatory and Th1 responses have also been found in infants with bronchiolitis. For example, the cytokines interferon- γ (IFN- γ), interleukin-2 (IL-2), IL-6, and IL-8, and chemokines macrophage inflammatory protein-1 α (MIP-1 α) and regulated upon activation, normal T cell expressed and secreted (RANTES) were found in nasal lavage and blood samples during RSV bronchiolitis [Abu-Harb et al., 1999; van Schaik et al., 1999; Brandenburg et al., 2000; Garofalo et al., 2001; Sung et al., 2001; Tripp et al., 2002]. During upper respiratory tract infection in infants, elevated levels of proinflammatory and Th1 cytokines IL-1 β , IL-6, IL-8, and tumour necrosis factor- α (TNF- α) have been observed in nasal samples [Noah et al., 1995]. Limited data are available comparing RSV-induced bronchiolitis and RSV-induced upper respiratory tract infection.

This study investigated whether differences could be observed on the cellular level between the nasal immune responses of infants with RSV-induced bronchiolitis and RSV-induced upper respiratory tract infection. For this purpose, the numbers of nasal inflammatory cells, the numbers of Th1- and Th2-like cytokine-positive cells, and the numbers of proinflammatory cytokine-positive cells were determined in both groups of children. The results show distinct nasal immunological effects during RSV-induced bronchiolitis versus RSV-induced upper respiratory tract infection.

MATERIALS AND METHODS

Patients and Study Design

During the winters of 1998 and 1999, 14 infants (median age 9 weeks) seen at the Sophia's Children Hospital in Rotterdam (The Netherlands) with RSV-induced lower respiratory tract infection (bronchiolitis) were selected for inclusion in the study. The diagnosis of bronchiolitis was based on the diagnosis of RSV infection in the presence of clinical symptoms and radiological findings characteristic for lower respiratory tract disease. In nearly all cases, the associated respiratory problems required hospitalisation of the affected children. Eight infants (median age, 26 weeks) were selected for comparison. They had participated in a prospective birth cohort study and presented with mild

upper respiratory tract infection symptoms caused by RSV without bronchiolitis. None of the children with upper respiratory tract infection went to hospital. Physical examination was performed and nasal brush samples were taken from the upper respiratory tract infection group within the first few days after the onset of infection (median, 3 days). This was done within 24 hr after arrival in the hospital for the bronchiolitis group. To obtain baseline measurements, nasal brush samples were taken from all infants with upper respiratory tract infection and from 11 of the 14 infants with bronchiolitis during the convalescent phase of the disease (2 to 4 weeks later). The study was approved by the Medical Ethical Committee of the Erasmus University Medical Centre Rotterdam. Written informed consent was given by all parents for participation of their child in this study.

Nasal Brushes and Viral Diagnostics

Cells were harvested from the nose with a cytobrush (Medscand Medical, Sweden) and processed as described elsewhere [Godthelp et al., 1996]. Cells were washed in RPMI 1640 medium (Life Technologies), and cytospin preparations were made on 10% (w/v) poly-L-lysine (Sigma)-coated microscope slides. RSV infection was confirmed by direct immunofluorescent staining of nasal brush cells with antiviral antibodies [Rothbarth et al., 1988] and/or by virus isolation from nasal brush supernatant.

Immunohistochemical Staining of CD3, CD68, MBP, ICAM-1, and IL-18

Slides were fixed in acetone and placed in a semi-automatic stainer (Sequenza, Shandon, Amsterdam, The Netherlands). Immunohistochemical staining was carried out as described elsewhere [Godthelp et al., 1996]. Briefly, slides were pre-incubated with 10% (v/v) normal goat serum (NGS; CLB, The Netherlands) (10 min) and subsequently for 60 min with mouse anti-human monoclonal antibodies directed against CD3, CD68, major basic protein (MBP), intercellular adhesion molecule-1 (ICAM-1), or IL-18 diluted in phosphate-buffered saline (PBS) supplemented with 1% (w/v) blocking reagent (Boehringer-Mannheim, Germany) (Table I). After 30-min incubation for with biotinylated goat anti-mouse Ig serum, slides were incubated for 30 min with either streptavidin alkaline phosphatase for CD3, CD68, and MBP or with polyclonal goat anti-biotin antibody for ICAM-1 and IL-18. After New Fuchsin (Chroma, Germany) staining, sections were counterstained with Gill's hematoxylin and were mounted in glycerin-gelatin. Isotypic control antibody was used for control staining.

Tyramide Signal Amplification (TSA) Staining for IL-4, IL-8, IL-10, IL-12, and IFN- γ

A sensitive protocol was used based on the alkaline phosphatase method described above. Slides were

TABLE I. Effects of Monoclonal Antibodies in RSV

Antibody	Specificity	Cell type/ cytokine	Concn ($\mu\text{g/ml}$)	Source
T3-4B5	CD3	T lymphocytes	4.2	DAKO, Denmark
EBM11	CD68	Macrophages	4.6	DAKO, Denmark
BMK-13	MBP	Eosinophils	0.2	Sanbio, The Netherlands
MEM-112	CD54	ICAM-1	144	Sanbio, The Netherlands
1-41-1	IL-4	IL-4	12	Novartis, Switzerland
B-E8	IL-6	IL-6	10	Bender Medsystems, Austria
NAP II	IL-8	IL-8	1	Bender Medsystems, Austria
IC25-471	IL-10	IL-10	10	Instruchemie, The Netherlands
24945.11	IL-12p70	IL-12	5	R&D systems, United Kingdom
500-M87	IL-18	IL-18	20	Peprotech, United Kingdom
MD-1	IFN- γ	IFN- γ	2.5	Innogenetic, Belgium

RSV, respiratory syncytial virus; IL-4, interleukin-4; IFN- γ , interferon- γ .

incubated with mouse anti-human monoclonal antibodies directed against IL-4, IL-8, IL-10, IL-12, IFN- γ , or an isotypic control antibody for 60 min (Table I). After incubation with biotinylated goat anti-mouse Ig serum, endogenous peroxidase was blocked using 0.2% (w/v) azide, 0.02% (v/v) hydrogen peroxide and 50% (v/v) methanol in PBS. Slides were then incubated with streptavidin conjugated peroxidase (30 minutes) (NEN, USA), biotinyl tyramide in Tris-HCl buffer (10 min) for amplification of the signal, with alkaline-phosphatase conjugated goat-anti-biotin and New Fuchsin substrate (30 min).

Immunohistochemical Staining of IL-6

Sections were stained for IL-6 (Table I) using the *polyMICA* immunohistochemical staining system of The Binding Site Ltd. (Birmingham, UK) in accordance with the manufacturer's instructions.

Light Microscope Evaluation

In this study, 1,000 cells stained with a purple-blue nucleus were counted in every nasal brush sample. All slides were blinded and counted by two independent investigators in order to ensure an objective analysis. The number of positively stained cells was calculated as a percentage of 1,000 nasal brush cells. CD3 and ICAM-1 positively stained cells had a red cell membrane. Red cytoplasmic staining was found for CD68, MBP, IL-4, IL-8, IL-10, IL-12, IL-18, and IFN- γ . IL-6 positive cells had dark brown cytoplasmic staining. On the basis

of morphology, both inflammatory and ciliated epithelial cells were found to stain positive for cytokines.

Statistical Analysis

Statistical analysis of cell numbers carried out with SPSS. Percentages of positive cells were log-transformed to obtain a normal distribution among all data. The paired sample *t*-test was used to analyse differences between the two sampling moments. Differences in cytokine-positive cells between patients with bronchiolitis and upper respiratory tract infection and between different patient characteristics were analysed with the independent sample *t*-test. Correlations between percentages of cells and the age of the child were tested using Spearman's correlation coefficient. Differences between patient groups and sampling moments were considered statistically significant when the *P* value was ≤ 0.05 .

RESULTS

Patient Characteristics

All but one of the 14 patients with RSV bronchiolitis needed hospital admission. Six infants were admitted to the medium-care unit and seven to the intensive care unit (ICU). Three of the infants admitted to the ICU required mechanical ventilation. All the bronchiolitis infants suffered from a runny nose and cough, and three infants had wheezing symptoms. None of the eight infants with mild RSV upper respiratory tract infection symptoms was admitted to the hospital. Patient characteristics are summarised in Table II.

TABLE II. RSV Patient Characteristics

	Bronchiolitis	URTI
No. of patients	14	8
Age (wk) ^a	9 (3–25)	26 (8–52)
Gender (male)	36%	63%
Smoking parent	36%	13%
ICU	50%	0%
Birth weight (g) ^a	3150 (2295–4300)	3880 (2300–4950)
Duration of pregnancy (wk) ^a	38 (36–42)	40 (38–42)

^aMedian (range).

RSV, respiratory syncytial virus; ICU, intensive care unit; URTI, upper respiratory tract infection.

Inflammatory Cellular Responses in RSV-Induced Upper Respiratory Tract Infection and Bronchiolitis

During the acute phase of bronchiolitis as well as during upper respiratory tract infection, there was a marked increase in the numbers of macrophages (CD68-positive cells) in nasal brush samples compared with convalescent samples (Fig. 1A). The median numbers increased from 0.9 to 4.4% in upper respiratory tract infection ($P=0.009$) and from 1.4 to 9.6% in bronchiolitis ($P=0.001$). Although the median number of macrophages in the acute phase was higher during bronchiolitis than during upper respiratory tract infection, this was not statistically significant. The influx of macrophages was paralleled by a similar influx of T lymphocytes (CD3-positive cells). Statistically significant increases in numbers of T lymphocytes were observed in upper respiratory tract infection (from median 1.7–2.3%; $P=0.02$), and a trend was also found toward increased numbers of T lymphocytes in bronchiolitis (from median 1.3–2.3%; $P=0.06$) (Fig. 1B). No differences were found between the two groups.

The recruitment of inflammatory cells to the site of infection is often accompanied by an increased local expression of adhesion molecules that facilitates the migration of these cells [Wang et al., 2000]. This also seems to be the case in this study. An increase was observed in the number of ICAM-1 positive cells during both upper respiratory tract infection (from median 11.7–18.4%) and bronchiolitis (from median 12.3–22.7%). However, compared with convalescence, significantly elevated numbers of ICAM-1 positive cells were only found during the acute phase of bronchiolitis ($P=0.04$) (Fig. 1C).

Small numbers of eosinophils (BMK-13 positive cells; median, 0%; range, 0–0.4%, Fig. 1D) were also found in nasal brush samples. No differences were observed in the number of eosinophils, either between acute and convalescent samples or between bronchiolitis and upper respiratory tract infection in the acute phase (Fig. 1D). However, during the convalescent phase in bronchiolitis, a small but significantly higher number of eosinophils were detected compared with the convalescent phase of patients presenting with upper respiratory tract infection (median 0% vs. 0.01%; $P=0.05$).

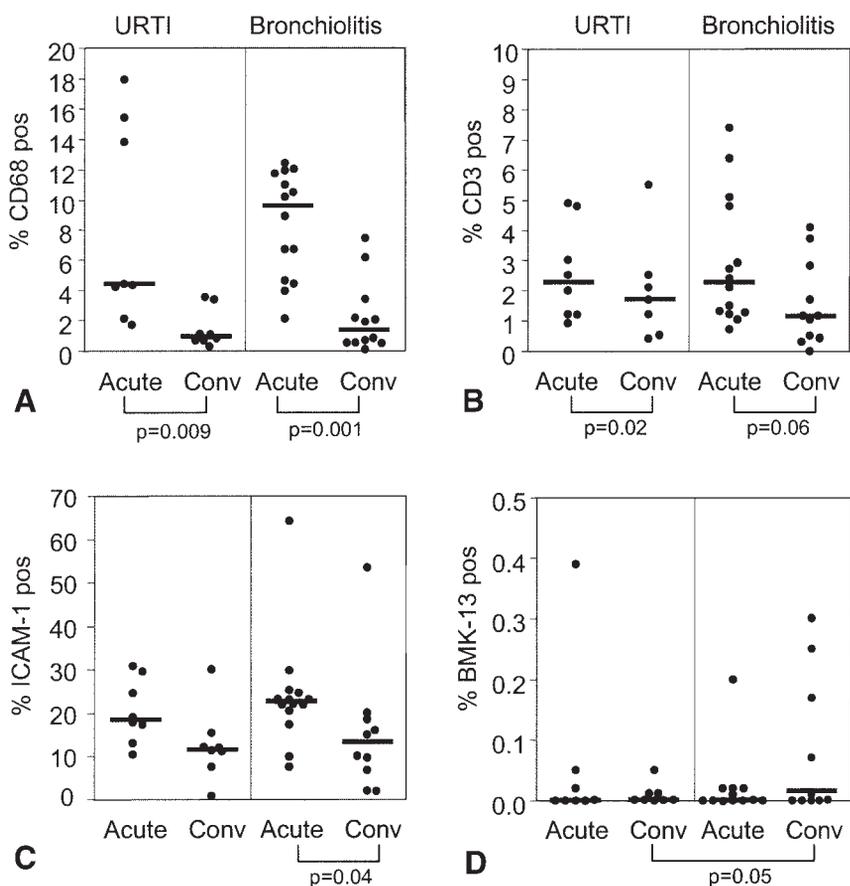


Fig. 1. Percentages of (A) macrophages (CD68-positive), (B) T lymphocytes (CD3-positive), (C) intercellular adhesion molecule-1 (ICAM-1)-positive cells, and (D) eosinophils (major basic protein [MBP]-positive) during the acute and convalescent phase (conv) of respiratory syncytial virus (RSV)-induced upper respiratory tract infection (URTI) and bronchiolitis. Bars represent median percentages.

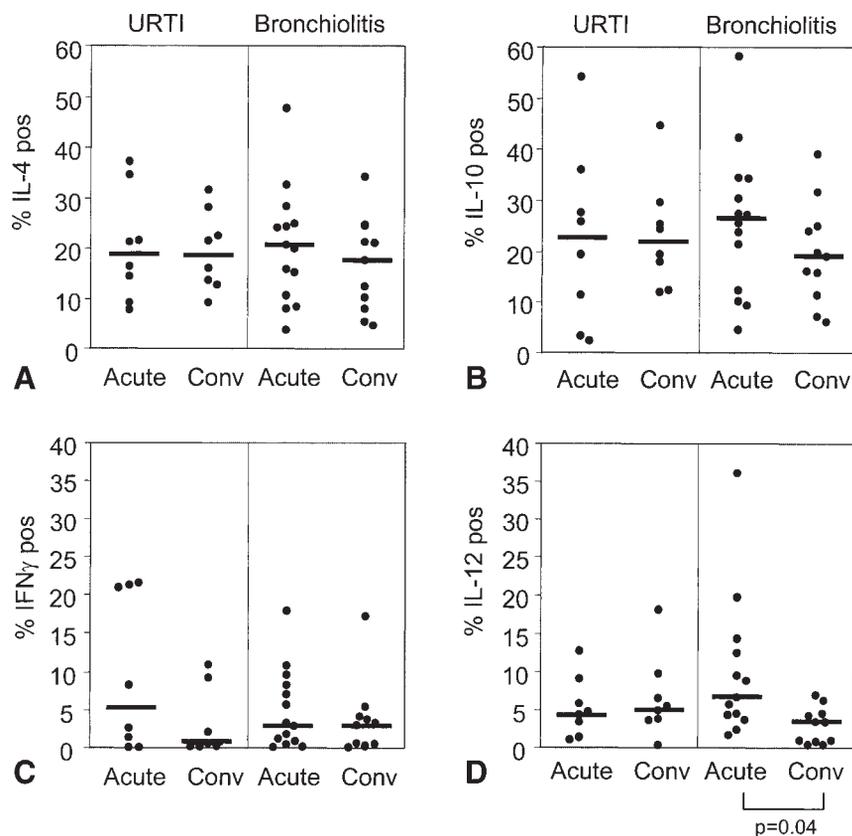


Fig. 2. Percentages of interleukin-4 (IL-4) (A), IL-10 (B), interferon- γ (IFN- γ) (C), and IL-12 (D) positive cells during the acute and convalescent phase (conv) of respiratory syncytial virus (RSV)-induced upper respiratory tract infection (URTI) and bronchiolitis. Bars represent median percentages.

Th1- and Th2-Like Cytokine-Positive Cells

Nasal brush samples were also stained for Th1-like cytokines IL-12 and IFN- γ , and for Th2-like cytokines IL-4 and IL-10 (Fig. 2). In bronchiolitis, median numbers of IL-12 positive cells increased from 3.2% at baseline (convalescence) to 6.6% during the acute phase of infection ($P=0.04$). There were no differences between bronchiolitis and upper respiratory tract infection in terms of numbers of IL-12 positive cells. By contrast, no differences were found for IL-4, IL-10 and IFN- γ positive cells between the acute and convalescent phase or between upper respiratory tract infection and bronchiolitis. Similarly, no differences were found in the Th2/Th1 ratios (IL-4/IFN- γ or IL-10/IL-12) for patients with RSV bronchiolitis and upper respiratory tract infection at either sampling time point. However, there was a fall in the IL-10/IL-12 ratio during the acute phase of bronchiolitis compared with the convalescent phase ($P=0.04$).

Proinflammatory Cytokines: IL-6, IL-8, and IL-18

In contrast with the Th1- and Th2-like cytokine positive cells, an increase in numbers of proinflammatory IL-18 positive cells was found during bronchiolitis compared with convalescence (from median 27.0–

68.5%; $P=0.01$; Fig. 3A). Interestingly, this increase was only observed during bronchiolitis, and not during upper respiratory tract infection. During the acute phase of infection, numbers of IL-18 positive cells were higher during bronchiolitis than upper respiratory tract infection ($P=0.001$).

Although children with bronchiolitis and with upper respiratory tract infection differed in age at the moment of sampling, the increase in numbers of IL-18 positive cells during bronchiolitis is not a consequence of the differences in age between the two groups. As shown in Figure 4, numbers of IL-18 positive cells did not relate to the age of the child during infection in either children with bronchiolitis or children with upper respiratory tract infection. During baseline (convalescence), no differences in numbers were observed between bronchiolitis and upper respiratory tract infection. Moreover, no age-dependent maturation was observed either. Among infants with bronchiolitis, numbers of IL-18 positive cells were not related to the severity of infection, as determined by the need for mechanical ventilation or admittance to the ICU.

In contrast to IL-18, the numbers of IL-6 positive cells increased for both bronchiolitis (from median 9.9–18.5%) and upper respiratory tract infection (from median 19.0–30.9%) (Fig. 3B). However, this increase was only statistically significant for bronchiolitis

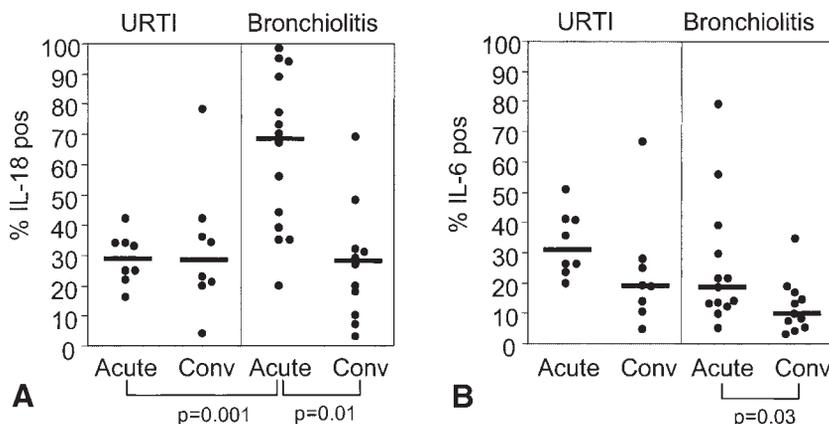


Fig. 3. Percentages of interleukin-18 (IL-18) (A) and IL-6 (B) positive cells during the acute phase and convalescent phase (conv) of respiratory syncytial virus (RSV)-induced upper respiratory tract infection (URTI) and bronchiolitis. Bars represent median percentages.

($P = 0.03$) but not for upper respiratory tract infection. No differences were observed in terms of IL-6 positive cells between bronchiolitis and upper respiratory tract infection during either the acute or convalescent phase. Median numbers of IL-8 positive cells ranged between 0.5% and 45.1%, and no differences were found between acute and convalescent sampling or between bronchiolitis and upper respiratory tract infection (data not shown).

DISCUSSION

During both RSV-induced upper respiratory tract infection and bronchiolitis, a general nasal inflammation was observed. This was characterised by increased numbers of macrophages, T lymphocytes, and ICAM-1 positive cells, a finding that is in line with previous observations [De Weerd et al., 1998; Grigg et al., 1999]. Most importantly, this study showed a striking difference in cytokine responses between both types of infection, i.e., an increase in the number of IL-18 positive

cells in nasal brush samples during bronchiolitis. This increase was not evident during upper respiratory tract infection. The strong IL-18 response was accompanied by an increase in proinflammatory cytokine IL-6 and Th1-like cytokine IL-12 responses during bronchiolitis, but these responses were not different from those in patients with upper respiratory tract infection.

IL-18 is a proinflammatory cytokine, and its effect is closely related to that of IL-1, i.e., the induction of TNF α , IL-1, IL-6, IL-8, granulocyte-macrophage-colony-stimulating factor (GM-CSF), ICAM-1, Fas ligand, and several chemokines and the inhibition of IL-10 and IgE production [Nakanishi et al., 2001; Wang et al., 2001]. IL-18 is also involved in antiviral mechanisms and is, in combination with IL-12, a powerful inducer of IFN γ production from T lymphocytes and natural killer (NK) cells [Dinarello, 1999]. IL-18 can therefore stimulate Th1 responses. In these nasal brush samples, an increase was observed in IL-18 and IL-12 responses during bronchiolitis, but this was not accompanied by a corresponding increase in the numbers of IFN- γ positive cells. Garofalo et al. [2001] did find higher levels of IFN- γ protein in nasopharyngeal samples of infants with RSV bronchiolitis compared with RSV-induced upper respiratory tract infection. It is possible that the increase in the expression of IFN- γ per cell was too small to be detected in this study due to the immaturity of the children's immune systems. This is supported by the observation that when mononuclear cells or T lymphocytes from cord blood are stimulated with mitogen, lower levels of IFN- γ protein are produced compared with cells isolated from adult blood [Chalmers et al., 1998; Pit et al., 2000]. However, the approach in the present study did not allow us to determine whether levels of IFN- γ expression rather than cell numbers could have been upregulated. Despite this uncertainty, the observation of increasing numbers of IL-18 and IL-12 positive cells during RSV-induced bronchiolitis could explain the underlying mechanism of increased IFN- γ production observed during general bronchiolitis in children [van Schaik et al., 1999].

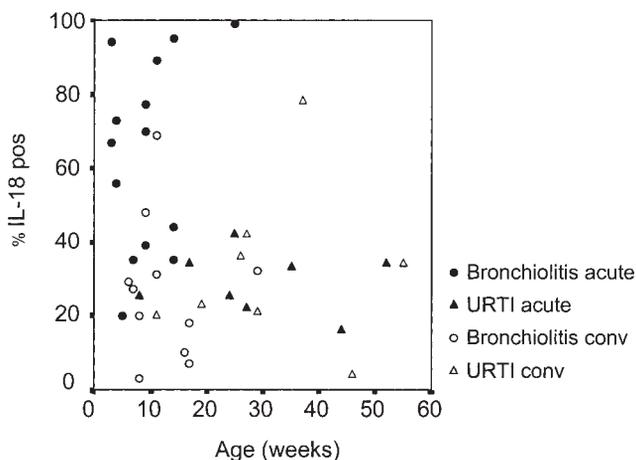


Fig. 4. Percentages of interleukin-18 (IL-18) positive cells related to the age of the child during the acute and convalescent (conv) phase of respiratory syncytial virus (RSV)-induced bronchiolitis or upper respiratory tract infection (URTI).

With respect to the other proinflammatory cytokines IL-6 and IL-8, there was no similar distinction between bronchiolitis and upper respiratory tract infection. Although IL-6 responses are increased during the acute phase of an RSV bronchiolitis compared with convalescence, this increase has also been observed for upper respiratory tract infection. There were no differences in the number of IL-8 positive cells. However, others have found increased levels of IL-8 protein in nasal samples during bronchiolitis [Abu-Harb et al., 1999]. Noah et al. [1995] found elevated protein levels for both cytokines in nasal pharyngeal samples of infants with upper respiratory tract infection. Furthermore, levels of IL-6 and IL-8 proteins in plasma were higher in infants with severe, as compared with mild, RSV infection [Bont et al., 1999; Brandenburg et al., 2000]. This discrepancy with the findings in this study is probably a consequence of the differences in the methods used to detect changes in the immune response. Where this study determined changes in cell numbers, the other studies examined protein levels.

Nasal brushes are easy to carry out in infants and can adequately document cellular immune responses in the nose. In these brushes, it was observed on the basis of morphology that cytokines are not only produced by inflammatory cells but also by ciliated epithelial cells. This is in line with other studies indicating that cytokines are produced by epithelial cells after infection with RSV [Arnold et al., 1994; Fujishima et al., 1998]. This explains the higher percentages of cytokine-positive cells observed in cytopins in the present study, as would be expected purely on the basis of the number of inflammatory cells present in such samples. A possible caveat of the study is the age difference between infants in the two groups, which could have skewed the results. Although cytokine responses could depend on the age of the child [Buck et al., 2002], no evidence was found that the IL-18 data were affected. As shown in the Figure 4, no age-related maturation of IL-18 responses was observed, either in infants with bronchiolitis or in infants with upper respiratory tract infection. Moreover, at baseline (convalescence), numbers of IL-18 positive cells did not differ between the two groups, and there was no age-related maturation. This shows that the differences in IL-18 response between the two groups are not due to differences in age, but rather that this response represents an increase in IL-18 positive cells in children with severe RSV-induced infection. Similarly, possible confounders, such as gender, birth weight, duration of pregnancy, cigarette smoking by the parents, and allergic disease in the family of the children, did not differ significantly between the groups and did not affect the results (data not shown).

It remains unclear whether the distinct nasal immune response for IL-18 in RSV-induced bronchiolitis is functionally relevant. It would be interesting to determine whether this nasal increase in IL-18 could be a direct reflection of the immune response in the lower airway during RSV bronchiolitis or the severity of infection. A potential link between IL-18 expression

and severity of disease has been postulated before. During tuberculosis, for instance, higher levels of IL-18 were found in patients with high-grade fever compared with patients without fever [Yamada et al., 2000]. An additional example of the link between IL-18 and severity of disease was observed in asthmatic patients. In patients with asthma, not only were IL-18 levels significantly higher in serum during an acute exacerbation than on remission days, but IL-18 levels were also related inversely to peak expiratory flow [Tanaka et al., 2001]. Although patients were excluded who were positive for some of the respiratory viruses (RSV, parainfluenza virus, influenza virus), the study did not look for the most prevalent inducers of respiratory infection and asthma exacerbations, namely rhinovirus and coronavirus [Nicholson et al., 1993; Makela et al., 1998]. It is therefore possible that the increase in levels of IL-18 during exacerbations is a direct reflection of a respiratory viral infection.

The increased IL-18 response during bronchiolitis could also be linked to the eosinophilia in nasal and peripheral blood samples. This has often been described as accompanying bronchiolitis [Garofalo et al., 1994; Ehlenfeld et al., 2000]. Until recently, the increase in numbers of eosinophils was thought to result from enhanced Th2 responses during infection [Roman et al., 1997]. The present study could not confirm this hypothesis, as no Th2-skewed response during bronchiolitis was observed. A previous study in our group showed that RSV-stimulated peripheral blood T lymphocytes taken during bronchiolitis produced predominantly Th1, and some Th2, cytokines, whatever the clinical severity of the underlying disease [Brandenburg et al., 2000]. Similar results were recently published by Tripp et al. [2002], who found an increase in both RSV-specific Th1 and Th2 cytokine-positive T lymphocytes during bronchiolitis compared with controls. On the other hand, the present data do suggest an alternative explanation for the observed eosinophil influx. IL-18 has been shown to induce the expression of the eosinophil chemoattractants RANTES and eotaxin [Campbell et al., 2000; Alaeddine et al., 2001]. In combination with the increase in levels of ICAM-1 expression by IL-18 [Yoshida et al., 2001], this cytokine could be responsible for the eosinophilia observed during and after bronchiolitis.

In conclusion, despite the limitations of this study, clear differences were found in the local nasal immune response of children presenting with either RSV-induced upper respiratory tract infection or bronchiolitis. RSV bronchiolitis was characterised by a strong increase in cells positive for the proinflammatory cytokine IL-18. The increase in IL-18 positive cells is specific for bronchiolitis, as a similar increase could not be detected in RSV-induced upper respiratory tract infection. Although IL-6 and IL-12 responses also increased during bronchiolitis, these were not different from patients with upper respiratory tract infection. The strong IL-18 responses during bronchiolitis could be a direct reflection of the severity of infection and could

explain the eosinophilia frequently observed during bronchiolitis.

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