Specific increase in local IL-17 production during recovery from primary RSV bronchiolitis

Faber, T.E.; Groen, H.; Welfing, M.; Jansen, K.J.G.; Bont, L.J.

Published in:
Journal of Medical Virology

DOI:
10.1002/jmv.23291

Journal of Medical Virology 84:1084–1088 (2012)

Specific Increase in Local IL-17 Production During Recovery From Primary RSV Bronchiolitis

Tina E. Faber,[1]* Henk Groen,[2] Martine Welfing,[1] Koos J.G. Jansen,[3] and Louis J. Bont[4]

[1]*Department of Pediatrics, Medical Center Leeuwarden, Leeuwarden, The Netherlands*
[2]*Department of Epidemiology, University Medical Center Groningen, Groningen, The Netherlands*
[3]*Pediatric Intensive Care Unit, Wilhelmina Children's Hospital, University Medical Center Utrecht, Utrecht, The Netherlands*
[4]*Department of Pediatric Infectious Diseases, University Medical Center Utrecht, Wilhelmina Children's Hospital, Utrecht, The Netherlands*

Although Respiratory syncytial virus (RSV) bronchiolitis is the most important cause of hospital admission for infants during the winter season, the pathogenesis is largely unknown. Interleukin-17 (IL-17) concentrations were studied in nasopharyngeal aspirates from 21 non-ventilated and 17 ventilated infants admitted to hospital with RSV bronchiolitis at time of admission and discharge from the hospital. On admission, nasopharyngeal concentrations of most cytokines and chemokines were lower in non-ventilated infants than in ventilated infants, reaching statistical significance for Eotaxin, IL-1α, and IL-6. During course of disease, nasopharyngeal concentrations of most cytokines and chemokines decreased, reaching statistical significance for IL-6 and IP-10. However, nasopharyngeal IL-17 concentrations were higher at discharge than at admission in children with non-ventilated RSV disease (209–101 pg/ml, $P = 0.008$), a response pattern not observed in ventilated RSV patients nor for other cytokines or chemokines. It is speculated that local IL-17 production may be involved during convalescence from RSV bronchiolitis in non-ventilated patients by facilitating innate and adaptive antiviral immune responses. The role of IL-17 in the pathogenesis of RSV bronchiolitis is to be explored further. ***J. Med. Virol. 84:1084–1088, 2012.***

KEY WORDS: respiratory syncytial virus; cytokines and chemokines; interleukin-17; innate immune responses

INTRODUCTION

Human respiratory syncytial virus (RSV) is a species in the genus *Pneumovirus*, family *Paramyxoviridae*. RSV bronchiolitis is the most common cause of hospitalization for infants during the winter season. Prematurity, chronic lung disease, congenital heart disease, Down's syndrome, and neuromuscular disease are conditions associated with severe course of disease [Welliver, 2003; Bloemers et al., 2007; Resch et al., 2009]. However, most children hospitalized for RSV bronchiolitis were healthy until infected. Viral loads have been associated with disease severity [DeVincenzo et al., 2005; Houben et al., 2010]. The precise mechanisms underlying human RSV bronchiolitis are largely unknown and most likely multi-factorial, in which both immunological and non-immunological responses, as well as genetic susceptibility play a role.

Interleukin-17 (IL-17) or IL-17A is a member of a family of cytokines with pro-inflammatory effector function. The IL-17 family, including IL-17A to IL-17F, represents a distinct signaling system and has been linked to many immune and auto-immune related diseases such as rheumatoid arthritis, lupus, allograft rejection and anti-tumor immunity, and specifically for IL-17F, allergic airway inflammation and asthma [Aggarwal and Gurney, 2002; Kawaguchi et al., 2009]. It was originally described as a product of Th17 cells, a distinct CD4+ T-cell subset bearing the IL-23 receptor [Korn et al., 2009]. It has now become clear that IL-17 is produced by various cells from the adaptive and innate immune system, such as

Grant sponsor: Small Grant Award from the European Society for Pediatric Infectious Diseases (ESPID).

None of the authors have a commercial or other association that might pose a conflict of interest.

*Correspondence to: Tina E. Faber, Pediatric Infectious Disease Specialist, Department of Pediatrics, Medical Center Leeuwarden, Henri Dunantweg 2, 8934 AD Leeuwarden, The Netherlands. E-mail: t.faber@umcutrecht.nl

Accepted 13 March 2012

DOI 10.1002/jmv.23291
Published online in Wiley Online Library (wileyonlinelibrary.com).

γδ-T-cells, Tc17 cells, and invariant natural killer T-cells (iNKT)-cells [Korn et al., 2009; Xie et al., 2009; Cua and Tato, 2010]. Although effector functions are incompletely understood, it is appreciated that IL-17 is capable of inducing a specific pro-inflammatory immune response through the production of many other cytokines, chemokines, and prostaglandins. During infection, IL-17 induces a tissue response required for clearance of bacteria, viruses, and fungi, possibly through induction of a neutrophil response [Onishi and Gaffen, 2010]. There are only limited data on the role of IL-17 in the defense against viral infections, in particular against RSV infection. In the current study, it was hypothesized that IL-17 production plays a role during convalescence of RSV disease. IL-17 was therefore studied during the acute phase and at recovery of disease in a cohort of infants hospitalized for RSV bronchiolitis. Differences in severity of disease was differentiated by need for mechanical ventilation. It was shown that local IL-17 production increases during recovery in children with hospitalized non-ventilated RSV bronchiolitis, suggesting that IL-17 may play a protective role against the development of severe RSV disease requiring mechanical ventilation.

MATERIALS AND METHODS

Patients

Children aged under 13 months with symptoms of lower respiratory tract infection admitted to the pediatric ward of two hospitals in The Netherlands were included during two consecutive winter seasons. Symptoms of lower respiratory tract infection were severe chest cough, wheezing, hoarseness, stridor, shortness of breath, cyanosis, and apnea. Children were included after nasopharyngeal aspirates were found positive for RSV by direct immunofluorescence, diagnosis was later confirmed by culture and polymerase chain reaction (PCR). Infants born prematurely with congenital heart disease or chronic lung disease, infants with wheezing illness before RSV bronchiolitis was diagnosed, and patients using corticosteroids or bronchodilators were not included. Differences in severity of RSV disease was differentiated by need for mechanical ventilation. All infants admitted to the pediatric intensive care unit were intubated and mechanically ventilated, therefore stay at the intensive care unit was identical to the requirement of mechanical ventilation. The study was performed in compliance with relevant laws and institutional guidelines and in accordance with the ethical standards of the Declaration of Helsinki and approved by the local Medical Ethics Committee at the Medical Center Leeuwarden. All parents or guardians gave written consent to participate in this study.

Nasopharyngeal Aspirates

Undiluted nasopharyngeal aspirates were taken within 24 hr after admission and at discharge from the hospital. Nasopharyngeal secretions were gently aspirated by an experienced physician with a 3.3 mm suction catheter, placed on ice immediately and stored at -80° for later analysis. Xylomethazolin or NaCl 0.9% nasal spray was not given in the 6 hr prior to aspiration. Cytokine and chemokine concentrations were measured in all aspirates using a highly specific multiplex assay [De Jager et al., 2003]. Sample collection and processing has been described previously [Bont et al., 2001; Schuurhof et al., 2011]. Samples were weighed, diluted as necessary, sonicated, centrifuged, and duplicate assays were performed on each. In addition to IL-17 (detection limit 10–5,000 pg/ml), the following cytokine and chemokine concentrations were measured: Eotaxin, IL-1α, IL-6, Interferon (IFN) inducible protein 10 (IP-10), IL-1β, Monokine induced by IFN-γ (MIG), macrophage inflammatory protein 1α (MIP-1α), soluble intercellular adhesion molecule 1 (sICAM1), and IFN-γ (detection limits 5–5,000 pg/ml).

Statistical Analysis

Paired cytokine and chemokine concentrations at time of admission in non-ventilated and ventilated infants were compared by Mann–Whitney U test. Mann–Whitney U test was also used to compare paired cytokine and chemokine concentrations at time of admission versus time of discharge from the hospital in both non-ventilated and ventilated patients. All tests of significance were two-sided. P-values < 0.05 were considered to be statistically significant.

RESULTS

The patients investigated consisted of 21 non-mechanically ventilated infants and 17 mechanically ventilated infants. Except for length of hospital stay, no significant differences were found in baseline characteristics between infants with or without need for mechanical ventilation. There was an equal distribution of male infants in both groups (43% vs. 59%). The median age at time of admission and the percentage of infants born prematurely before 37 weeks gestation was similar in non-ventilated and ventilated infants (14 vs. 9 weeks, and 14% vs. 29%, respectively). In both groups, a similar number of children had been ill $\geq$3 days before admission (76% vs. 53%). Median length of hospital stay was significantly longer in children with severe RSV disease requiring mechanical ventilation (6 days vs. 10 days; Table I). Concentrations of IFN-γ were below the level of detection in all samples. For each inflammatory mediator, analyses were made comparing time of admission to discharge in non-ventilated as well as ventilated patients. Furthermore, cytokine and chemokine concentrations at time of admission were compared in both groups. Finally, an attempt was made to analyze emerging patterns during course of disease. At time of admission, there was no difference in IL-17 concentration between non-ventilated and ventilated infants (101 pg/ml vs. 72 pg/ml; not significant). Median

TABLE I. Subject Characteristics

	Non-ventilated infants (n = 21)	Ventilated infants (n = 17)	Non-ventilated vs ventilated infants
Number of male infants	9 (43%)	10 (59%)	NS[a]
Prematurity (amenorrhoe <37 weeks of gestation)	3 (14%)	5 (29%)	NS[a]
Age at admission (median in weeks after birth (CI))	14 (13–26)	9 (6–29)	NS[b]
Symptoms ≥3 days before admission	16 (76%)	9 (53%)	NS[a]
Length of hospital stay (median in days (min-max))	6 (3–13)	10 (5–28)	$P < 0.001$[b]
Length of ventilation (median in days (min-max))	NA	9 (5–16)	

NS, not significant; NA, not applicable to non-ventilated infants.
[a]Fisher's exact test;
[b]Mann–Whitney U test.

nasopharyngeal concentrations of Eotaxin, IL-1α, and IL-6 were lower in non-ventilated patients (18 vs. 118 pg/ml, 368 vs. 4,001 pg/ml, 362 vs. 12,500 pg/ml, respectively; $P < 0.001$). IL-17 concentrations were significantly higher at discharge than at admission (209 pg/ml vs. 101 pg/ml; $P = 0.0083$) in non-ventilated infants. IL-17 concentrations did not increase during course of disease in ventilated infants (Fig. 1). Concentrations of other cytokines and chemokines followed equal patterns in both groups, and either decreased ($P < 0.05$ for IL-6 and IP-10) or remained stable ($P \geq 0.05$ for Eotaxin, IL-1α, IL-1β, MIG, MIP1α, and sICAM1) during hospitalization (Table II). In both ventilated and non-ventilated infants, length of hospital stay was not correlated to IL17 concentration at discharge. Furthermore, length of hospital stay was not correlated to the difference in IL17 concentration between admission and discharge. Age at admission was not correlated to IL17 concentration at time of admission nor at time of discharge.

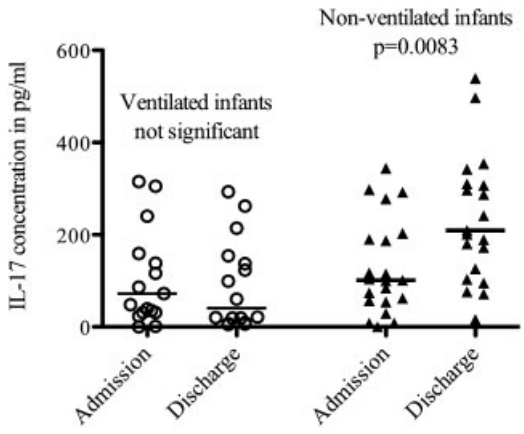

Fig. 1. Local IL-17 concentration increases during course of disease in non-ventilated infants with RSV bronchiolitis. IL17 concentration increases from time of admission to discharge from the hospital in 19 out of 21 (90%) non-ventilated children hospitalized for RSV bronchiolitis (median 101–209 pg/ml; $P = 0.0083$) and remains stable in ventilated infants (median 72–41 pg/ml; not significant).

DISCUSSION

In this study, local IL-17 production increased during the course of disease in non-ventilated infants with RSV bronchiolitis. Other inflammatory mediators either decreased or remained stable during course of disease in both ventilated and non-ventilated infants. These results could suggest that local IL-17 may play a protective role against the development of severe RSV bronchiolitis requiring mechanical ventilation. Furthermore, it implies that mechanisms that determine disease severity are apparently different than those that are involved in resolution of disease.

At time of admission, children requiring mechanical ventilation for RSV disease had higher levels of Eotaxin, IL-1α, and IL-6. Although it is not known whether cytokine concentrations are effected by mechanical ventilation, these results are in accordance with previous research showing high initial concentrations of pro-inflammatory mediators in children with most severe disease [Sheeran et al., 1999; Garafalo et al., 2001; Smyth et al., 2002; Welliver et al., 2002; McNamara et al., 2004]. Therefore, this study confirms that development of more severe RSV bronchiolitis is associated with a more robust cascade of inflammatory responses. Because most of these mediators can be produced by airway epithelium in response to RSV infection, this observation suggests, as is generally accepted in the case of RSV, a critical role for the epithelium as a first line of defence [McNamara et al., 2003].

IL-17 is a pleiotropic cytokine, originally thought to be produced only by T-cells, but is now known to be produced by innate immune cells, such as dendritic cells, iNKT and as γδ-T-cells. The IL-17 receptor is expressed ubiquitously in many tissues, including the lungs [Cua and Tato, 2010]. The role of IL-17 in host defence against viruses is understood incompletely. In general, antiviral defence depends heavily on type I IFNs, which are not known to be regulated by IL-17. Nevertheless, during experimental influenza infection, IL-17 production by CD8+ T-cells protects mice against disease and death [McKinstry et al., 2009; Xie et al., 2009; Onishi and Gaffen, 2010]. In this study, IL-17 increases during the course of disease in

TABLE II. Chemokine and Cytokine Analysis of Nasopharyngeal Aspirates of Ventilated and Non-Ventilated Infants With RSV Bronchiolitis During Course of Disease

	Non-ventilated infants		Ventilated infants		Analyses			
	Admission (1)	Discharge (2)	Admission (3)	Discharge (4)	*P* (1) vs (2)	*P* (3) vs (4)	*P* (1) vs (3)	Pattern
IL-17	101*	209	72	41	<0.05	NS	NS	↑
	56–197#	115–326	31–200	12–150				
IL-6	362	47	12,500	6,666	<0.001	<0.05	<0.001	↓
	107–2,021	26–97	6,666–24,750	2,130–6,666				
IP10	325	132	221	121	<0.05	<0.05	NS	↓
	201–453	1–278	142–201	65–251				
Eotaxin	18	22	118	137	NS	NS	<0.001	→
	11–50	15–56	56–277	73–338				
IL-1α	368	248	4,001	3,091	NS	NS	<0.001	→
	94–868	49–1,629	2,080–9,703	1,519–7,048				
IL-1β	132	130	203	111	NS	NS	NS	→
	62–176	64–282	79–254	68–177				
MIG	1,173	574	1,463	1,018	NS	NS	NS	→
	545–2,229	213–1,935	788–2,272	477–1,618				
MIP-1α	2,243	2,382	2,325	2,499	NS	NS	NS	→
	1,097–3,163	845–4,201	1,949–3,218	1,172–4,065				
sICAM	40,459	40,986	46,820	32,982	NS	NS	NS	→
	27,889–49,966	27,117–59,873	34,314–59,871	19,303–52,253				

NS, not significant.
Cytokine concentrations in median* and quartiles# in pg/ml.
In contrast to local IL17 concentrations which increase (↑) during course of disease in non-ventilated children with RSV (specifically $P = 0.0083$), other inflammatory mediators show the same pattern in non-ventilated and ventilated children and either decrease (↓) during course of disease, such as IL-6 and IP10, or remain stable (→) such as Eotaxin, IL-1α, IL-1β, MIG, MIP-1α and sICAM.

children with less severe RSV disease. This is in accordance with a recent study in which plasma IL-17 could not be detected in healthy children, and the highest concentration was found in moderately ill patients with RSV bronchiolitis when compared with severe cases [Larrañaga et al., 2009]. The source of IL-17 and the precise mechanism underlying local IL-17 production are not known and it remains unclear why the IL-17 response does not occur in ventilated children. It can be considered that adaptive immune cells, such as γδ-T-cells or Tc17 cells, are the source of increased IL-17 production. Another study did show increased IL-17 levels in tracheal aspirate samples from severely ill infants with RSV infection [Mukherjee et al., 2011]. The power of our study may have been too small to detect differences in this subpopulation. In addition, these children are generally younger and they may have different mechanisms of viral clearance. Also, it is not known whether intubation and mechanical ventilation itself have an effect on production of IL-17.

This study is the first attempt to measure local cytokine profiles during the course of disease in ventilated and non-ventilated patients with RSV bronchiolitis. A unique response pattern of IL-17 was identified which contrasts with other pro-inflammatory cytokines and chemokines that are associated with initial disease severity and generally decrease during the course of disease. In addition, the use of nasopharyngeal aspirate allowed for a measurement of absolute cytokine concentrations, which is not possible using other techniques, such as nasal washes. And although the association between nasopharyngeal aspirate cytokine concentrations and endotracheal cytokine concentrations is high [Joshi et al., 1998; Bont et al., 2001] there are unknown variables to be kept in mind when interpreting results that may influence mucus secretion, such as mechanical ventilation and respiration rate. Also, as the time between admission and discharge and hence the time between measurements is shorter in non-ventilated patients when compared to ventilated patients, this may potentially introduce a systemic error. Cytokine and chemokine response is a dynamic process and differences in sampling time may have a distorting effect. A further study limitation is the absence of proof that the increase in local IL-17 levels during the course of RSV bronchiolitis is causally related to less severe disease. In addition, the relatively small study population did not allow for extensive regression analysis to study potential confounders, in particular age, premature birth, length of hospital stay, and mechanical ventilation. Yet it is reassuring that length of hospital stay is not correlated to IL-17 concentration at discharge, nor to differences in concentration between admission and discharge. And although age at admission is an important variable to consider, it was not correlated to IL-17 concentration at time of admission nor at time of discharge. Another limitation in this study is not to have considered the quantification of viral load from respiratory secretions that would have allowed correlation of clinical and immunologic data.

In conclusion, IL-17 levels in the airways of RSV bronchiolitis patients increase during the course of

disease in hospitalized non-ventilated patients, suggesting a role in controlling of the disease. Further identification of the role of IL-17 in recovery from RSV bronchiolitis may lead to further insights into pathogenesis of disease.

REFERENCES

Aggarwal S, Gurney AL. 2002. IL-17: Prototype member of an emerging cytokine family. J Leukoc Biol 71:1–8.

Bloemers BL, van Furth AM, Weijerman ME, Gemke RJ, Broers CJ, van den Ende K, Kimpen JL, Strengers JL, Bont LJ. 2007. Down syndrome: A novel risk factor for respiratory syncytial virus bronchiolitis—A prospective birth-cohort study. Pediatrics 120:1076–1081.

Bont L, Heijnen CJ, Kavelaars A, van Aalderen WM, Brus F, Draaisma JM, Pekelharing-Berghuis M, van Diemen-Steenvoorde RA, Kimpen JL. 2001. Local interferon-gamma levels during respiratory syncytial virus lower respiratory tract infection are associated with disease severity. J Infect Dis 184: 355–358.

Cua DJ, Tato CM. 2010. Innate IL-17-producing cells: The sentinels of the immune system. Nat Rev Immunol 10:479–489.

De Jager W, te Velthuis H, Prakken BJ, Kuis W, Rijkers GT. 2003. Simultaneous detection of 15 human cytokines in a single sample of stimulated peripheral blood mononuclear cells. Clin Diagn Lab Immunol 10:133–139.

DeVincenzo JP, El Saleeby CM, Bush AJ. 2005. Respiratory syncytial virus load predicts disease severity in previously healthy infants. J Infect Dis 191:1861–1868.

Garafalo RJ, Patti J, Hintz KA, Hill V, Ogra PL, Welliver RC. 2001. Macrophage inflammatory protein 1α (not T-helper type 2 cytokines) is associated with severe forms of respiratory syncytial virus bronchiolitis. J Infect Dis 184:393–399.

Houben ML, Coenjaerts FE, Rossen JW, Belderbos ME, Hofland RW, Kimpen JL, Bont L. 2010. Disease severity and viral load are correlated in infants with primary respiratory syncytial virus infection in the community. J Med Virol 82:1266–1271.

Joshi P, Kakakios A, Jayasekera J, Isaacs D. 1998. A comparison of IL-2 levels in nasopharyngeal and endotracheal aspirates of babies with respiratory syncytial virus bronchiolitis. J Allergy Clin Immunol 102:618–620.

Kawaguchi M, Fujita J, Kokubu F, Huang SK, Homma T, Matsukura S, Adachi M, Hizawa N. 2009. IL-17F-induced IL-11 release in bronchial epithelial cells via MSK1-CREB pathway. Am J Physiol Lung Cell Mol Physiol 296:804–810.

Korn T, Bettelli E, Oukka M, Kuchroo VK. 2009. IL-17 and Th17 Cells. Annu Rev Immunol 27:485–517.

Larrañaga CL, Ampuero SL, Luchsinger VF, Carrión FA, Aguilar NV, Morales PR, Palomino MA, Tapia LF, Avendaño LF. 2009. Impaired immune response in severe human lower tract respiratory infection by respiratory syncytial virus. Pediatr Infect Dis J 28:867–873.

McKinstry KK, Strutt TM, Buck A, Curtis JD, Dibble JP, Huston G, Tighe M, Hamada H, Sell S, Dutton RW, Swain SL. 2009. IL-10 deficiency unleashes an influenza-specific Th17 response and enhances survival against high-dose challenge. J Immunol 182: 7353–7363.

McNamara PS, Ritson P, Selby A, Hart CA, Smyth RL. 2003. Bronchoalveolar lavage cellularity in infants with severe respiratory syncytial virus bronchiolitis. Arch Dis Child. 88:922–926.

McNamara PS, Flanagan BF, Selby AM, Hart CA, Smyth RL. 2004. Pro- and anti-inflammatory responses in respiratory syncytial virus bronchiolitis. Eur Respir J 23:106–112.

Mukherjee S, Lindell DM, Berlin AA, Morris SB, Shanley TP, Hershenson MB, Lukacs NW. 2011. IL-17-induced pulmonary pathogenesis during respiratory viral infection and exacerbation of allergic disease. Am J Pathol 179:248–258.

Onishi RM, Gaffen SL. 2010. Interleukin-17 and its target genes: Mechanisms of interleukin-17 function in disease. Immunol 129: 311–321.

Resch B, Manzoni P, Lanari M. 2009. Severe respiratory syncytial virus (RSV) infection in infants with neuromuscular diseases and immune deficiency syndromes. Paediatr Respir Rev 10:148–153.

Schuurhof A, Janssen R, de Groot H, Hodemaekers HM, de Klerk A, Kimpen JL, Bont L. 2011. Local interleukin-10 production during respiratory syncytial virus bronchiolitis is associated with post-bronchiolitis wheeze. Respir Res 12:121–123.

Sheeran P, Jafri H, Carubelli C, Saavedra J, Johnson C, Krisher K, Sánchez PJ, Ramilo O. 1999. Elevated cytokine concentrations in the nasal pharyngeal and tracheal secretions of children with respiratory syncytial virus disease. Pediatr Infect Dis 18:115–122.

Smyth RL, Mobbs KJ, O'Hea U, Ashby D, Hart CA. 2002. Respiratory syncytial virus bronchiolitis: Disease severity, interleukin-8, and virus genotype. Pediatr Pulmonol 33:339–346.

Welliver RC. 2003. Review of epidemiology and clinical risk factors for severe respiratory syncytial virus (RSV) infection. J Pediatr 143:112–117.

Welliver RC, Garofalo RP, Ogra PL. 2002. Beta-chemokines, but neither T helper type 1 nor T helper type 2 cytokines, correlate with severity of illness during respiratory syncytial virus infection. Pediatr Infect Dis J 21:457–461.

Xie D, Hai B, Xie X, Liu L, Ayello J, Ma X, Zhang J. 2009. Peripheral CD4+CD8+cells are the activated T cells expressed granzyme B (GrB), Foxp3, interleukin 17 (IL-17), at higher levels in Th1/Th2 cytokines. Cell Immunol 259:157–164.