Monocyte Chemoattractant Protein-1 and Interleukin-8 Are Increased in Bronchopulmonary Dysplasia: Relation to Isolation of Ureaplasma Urealyticum

R. John Baier, John Loggins, and Thomas E. Kruger

ABSTRACT

Background: **An exaggerated inflammatory response occurs in infants who subsequently develop bronchopulmonary dysplasia (BPD). *Ureaplasma urealyticum* (Uu) is frequently isolated from cultures of tracheal secretions obtained from very low birth weight infants and is associated with an increased risk of BPD.**

Methods: **We examined the relationships between isolation of genital mycoplasmas, tracheal aspirate (TA) interleukin-8 (IL-8), and monocyte chemoattractant protein-1 (MCP-1) concentrations and the development of BPD. Serial TAs were obtained prospectively from 35 very low birth weight infants, and IL-8 and MCP-1 concentrations were determined by enzyme-linked immunoadsorbent assay. Tracheal cultures for bacteria and genital mycoplasmas were performed on aspirates obtained during the first 2 days of life.**

Results: **Infants who developed BPD (n=18) were less mature (25.2±0.2 vs 27.8±0.5 weeks; $P<0.001$), of lower birth weight (746±28 vs 1052±41 g; $P<0.001$), and more likely to have a positive tracheal culture for Uu (39% vs 6%; $P=0.026$) than those who did not develop BPD (n=17). Tracheal concentrations of IL-8 and MCP-1 were significantly increased in infants who developed BPD (IL-8: $P=0.0001$; MCP-1: $P<0.001$, analysis of variance) and correlated with duration of mechanical ventilation and oxygen treatment. Uu-positive infants had an increased incidence of BPD (88% in infants with Uu vs 42% in infants without Uu; $P=0.020$) and had TA concentrations of IL-8 and MCP-1 that were significantly increased compared with those of Uu-negative infants.**

Conclusions: **Increased TA concentrations of IL-8 and MCP-1 during the first 2 weeks of life are associated with the development of BPD. Recovery of Uu from TAs is associated with a more robust inflammatory reaction and an increased risk of BPD. (J Investig Med 2001;49:362–369) Key Words: monocyte chemoattractant protein-1 • interleukin-8 • bronchopulmonary dysplasia • *Ureaplasma urealyticum***

INTRODUCTION

An exaggerated inflammatory response occurs in the first few days of life in infants who subsequently develop bronchopulmonary dysplasia (BPD). This response includes increases in airway protein concentration, inflammatory cells, leukotrienes, and cytokines.[1–11] Increased concentrations of interleukin-1β (IL-1β), interleukin-6 (IL-6), macrophage inflammatory protein-1α, tumor necrosis factor-α (TNFα) and interleukin-8 (IL-8) have been found in the tracheal aspirates (TA) of infants who developed BPD.[2,5–11] IL-8, a potent neutrophil chemoattractant and activator, is involved in the early stages of BPD development.[11]

During the evolution of the disease, TA cytology changes from that of neutrophil predominance to a predominance of macrophages.[1,2] Recruitment of mononuclear cells to the lung and their subsequent activation is regulated by chemotactic cytokines such as monocyte chemoattractant protein-1 (MCP-1).[12] MCP-1 has been implicated in the pathogenesis of other chronic inflammatory lung diseases that have a fibrotic component.[13–17] The role of MCP-1 or other monocyte chemotactic factors in BPD remains undefined.

From the Department of Pediatrics, Louisiana State University Health Sciences Center, Shreveport, La.

Address correspondence to: John Baier, MD, Department of Pediatrics, Louisiana State University Health Sciences Center, 1501 Kings Highway, Shreveport, LA 71130-3932. E-mail jbaier@lsuhsc.edu

Pulmonary infection is a potent stimulus for the production of cytokines in the lung.[18] In the early neonatal period, *Ureaplasma urealyticum* (Uu) is frequently isolated from cultures of tracheal secretions obtained from very low birth weight infants.[19,20] Isolation of Uu from the airways of mechanically ventilated very low birth weight infants is associated with increased TA cytokine concentrations and an increased risk of BPD.[19–23] It is therefore likely that infection with Uu causes an increase in cytokine production and the subsequent inflammatory response, thereby increasing lung injury.

We designed a prospective study to determine whether TA concentrations of IL-8 and MCP-1 are increased in infants who develop BPD during the first 3 weeks of life. Furthermore, we determined whether increases in these cytokines correlated with the isolation of Uu or *Mycoplasma hominis* (Mh) from TA.

METHODS

The study population consisted of 35 very low birth weight infants admitted to the neonatal intensive care unit at the Louisiana State University Health Sciences Center in Shreveport between July 1997 and March 1998. Eligibility criteria included a birth weight of less than 1500 g, mechanical ventilation (MV) during the first day of life, and informed consent. Infants who were not expected to survive 24 hours and those with major congenital anomalies were excluded. The study was approved by the Institutional Review Board for Human Research at Louisiana State University Health Sciences Center.

TA were collected only when suctioning was clinically indicated. Sterile normal saline (0.5 mL) was instilled into the endotracheal tube, and three to four manual breaths were provided by a self-inflating bag. Airway secretions were aspirated into sterile traps, and any material remaining in the catheter was washed into the trap with an additional 0.5 mL of saline. Serial aspirates were obtained at 0 to 24 hours, 48 to 72 hours, 4 to 5 days, 7 to 9 days, 12 to 14 days, and on day 21 if the infant remained on MV. Routine bacterial and genital mycoplasma cultures were performed on TA collected in the first 24 hours. Viral cultures were not routinely performed. The TAs were centrifuged 14,000 g for 10 minutes and the supernatant frozen at −20°C until assayed for cytokines by enzyme-linked immunoadsorbent assay (ELISA).

Clinical management of the infants was directed by the attending neonatologists. The use and timing of corticosteroids, treatment of patients who had positive TA cultures for genital mycoplasmas, and use of indomethacin prophylaxis were not specified by the protocol. Demographic data collected included the following: the need for surfactant therapy, duration of MV, use of corticosteroids, incidence of patent ductus arteriosus, use of indomethacin (prophylactic and therapeutic), presence and severity of intraventricular hemorrhage, and the presence of periventricular leukomalacia. Maternal data collected included mode of delivery, complications of pregnancy, and the use of antenatal corticosteroids and antibiotics. BPD was defined as oxygen dependency at 28 days postnatal age and a chest radiograph showing changes compatible with BPD.[24]

Genital Mycoplasma Culture

TA were cultured for the presence of both Mh and Uu using the following selective growth media: SP4 with urea (Remel, Lexana, Kan), Mycotrim GU® system (Irvine Scientific, Santa Anna Calif), as well as urea and arginine-enriched pleuropneumonia-like organism broths.[25] Fifty microliters of TA was cultured in 2 mL of the appropriate broths. An additional 50 μL of TA was placed into a Mycotrim flask and subsequently incubated at 37°C in 5% CO_2. The media was inspected twice a day for the first 2 days and then inspected daily for 5 days to observe any color change in the media indicative of a positive culture. Mycoplasma identification was confirmed by microscopic examination of the distinct colony characteristics of mycoplasmas on the solid media in the Mycotrim flask.

ELISA

Initial concentrations of all antibodies were used according to manufacturers' recommendations. Concentrations of antibodies were also titrated to ensure maximum sensitivity. Recombinant human cytokines IL-8 and MCP-1 and monoclonal and biotinylated monoclonal antibodies to IL-8 were purchased from Endogen (Woburn, Mass). Monoclonal antibody and biotinylated polyclonal antibody to MCP-1, as well as streptavidin-horseradish peroxidase conjugate (Strep-HRP), were purchased from Pharmingen (San Diego, Calif). Horseradish peroxidase-conjugated rabbit antigoat antibody, monoclonal and polyclonal goat antihuman secretory component antibodies, bovine serum albumin, and oxalic acid were purchased from Sigma Chemical Co (St Louis, Mo). Human secretory immunoglobulin A (IgA) was purchased from ICN Biomedicals (Costa Mesa, Calif). ELISA plates were obtained from Costar (Cambridge, Mass). 2,2'-azino-di[3-ethyl-benzthiazoline] peroxidase substrate kits were purchased from Bio-Rad (Hercules, Calif).

ELISA plates were coated with 100 μL of the appropriate monoclonal antibody in 0.1 mM sodium carbonate buffer (pH 9.6) and incubated overnight at 4°C. The monoclonal antibodies were coated at the following concentrations: MCP-1, 3.3 μg/mL; IL-8, 3.7 μg/mL; and

secretory component, 2.0 μg/mL. After coating, the ELISA plates were washed three times (5 minutes) with 200 μL of phosphate-buffered saline containing 0.05% Tween 20 (wash buffer). All subsequent washes were performed similarly. The plates were then blocked using 200 μL of phosphate-buffered saline/Tween 20 containing 3% (w/v) bovine serum albumin (blocking buffer) overnight at 4°C. After blocking, the plates were washed three times before use. All subsequent steps were performed in blocking buffer. TA and recombinant cytokine standards were appropriately diluted in blocking buffer.

For the IL-8 ELISA, 50 μL of biotinylated monoclonal antibody to IL-8 (540 ng/mL) was incubated with either 50 μL of diluted TA (diluted at least 1:10 in blocking buffer) or recombinant cytokine standards (3.9–1000 pg/mL) for 2 hours at room temperature. Plates were washed four times and incubated with Strep-HRP (1:1000) for 30 minutes at room temperature. Plates were washed four times as described above. The enzyme substrate, 2,2'-azino-di[3-ethyl-benzthiazoline] was used to quantify the amount of IL-8. The reaction was stopped with 100 μL of 2% oxalic acid and read at 405 nm using an ELISA autoreader (Cayman Chemical, Ann Arbor, Mich).

For the MCP-1 ELISA, 100 μL of diluted TA (diluted at least 1:20 in blocking buffer) or standards (3.9–1000 pg/mL) was incubated overnight at 4°C. Plates were washed four times and incubated with 100 μL of biotinylated polyclonal antibody (500 ng/mL) for 2 hours at room temperature. Plates were washed six times and then incubated with Strep-HRP (1:1000) for 30 minutes at room temperature. After washing six times, plates were developed as described above.

For the secretory component of IgA ELISA, secretory IgA was used as the standard. One hundred microliters of diluted TA (diluted 1:400–1:6400 in blocking buffer) or human secretory IgA standards (1.64–210 ng/mL) were incubated for 2 hours at room temperature. Plates were washed three times as described above and then incubated with polyclonal goat antihuman secretory component antibody overnight at 4°C. Plates were washed three times and incubated with HRP-conjugated rabbit-antigoat antibody (1:1000) for 2 hours at room temperature. After three washes, plates were developed as previously described. Sensitivities of the assays were as follows: IL-8, 15 pg/mL; MCP-1, 8 pg/mL; secretory component, 3 ng/mL.

Data Analysis

To adjust for variation in collection of TA, IL-8 and MCP-1 concentrations were normalized to secretory component of IgA.[26] All statistical analyses were performed using the SPSS for Windows version 6.0 (SPSS, Inc, Chicago, Ill). χ^2 analysis was used to assess the statistical differences in categorical variables. The Student's *t* test was used to assess normally distributed variables. The duration of MV, oxygen use, and hospitalization were not normally distributed, hence the Wilcox rank sum test was used for analysis of these factors. Similarly, tracheal cytokine concentrations were not normally distributed and were log-transformed before analysis. The differences in TA cytokine concentrations were assessed using analysis of variance (ANOVA). Multiple logistic regression using backward stepwise elimination of variables was used to determine which factors were associated with the development of BPD. The factors analyzed by logistic regression were as follows: birth weight, gestational age, maximum IL-8 concentration, maximum MCP-1 concentration, and tracheal Uu culture results. A probability value of less than 0.05 was considered statistically significant. The data are presented as mean±SEM.

RESULTS

Of the 35 patients enrolled in the study, 18 (51%) developed BPD and 17 (49%) did not develop the disease. Infants who developed BPD were smaller, of lower gestational age, more likely to have a TA culture positive for Uu, and more likely to have received treatment with indomethacin or postnatal dexamethasone (Table 1). There were three deaths among the study patients. One infant who was included in the BPD group died at 14 days of age from pulmonary hemorrhage, Uu pneumonia, and progressive respiratory failure. Another patient in the BPD group died of respiratory failure as a result of Uu infection, pulmonary hemorrhage, and fungal sepsis at 43 days of age. A third patient with BPD died of sepsis and multiorgan failure at 49 days of age.

One hundred thirty-four TAs were obtained during the first 3 weeks of life from study infants. Infants who did not develop BPD were extubated before day 21; therefore, only TA cytokine concentrations obtained before day 21 were used for analysis of differences for this outcome. IL-8 was detectable in all TA samples. TA concentrations of IL-8 rose early in the course of hyaline membrane disease, with maximum concentrations occurring between days 2 and 3 in the group who developed BPD (Figure 1A). TA concentrations of IL-8 were significantly higher in infants who developed BPD ($P=0.0001$, ANOVA). Moreover, TA IL-8 concentrations in those infants who developed BPD remained elevated for the 2-week period studied. In comparison, infants who did not develop BPD had TA IL-8 concentrations that returned to baseline levels by 4 to 5 days. Maximum IL-8 concentrations were significantly higher in infants who subsequently developed BPD as opposed to those who did not (596±165 pg/μg/s vs 105±28 pg/μg/s; $P=0.0090$).

Table 1. Clinical characteristics of study subjects.

Characteristic	BPD (n=18)	No BPD (n=17)	*P*
Birth weight, g	746±2	1052±42	<0.001
Gestation, weeks	25.2±0.2	27.8±0.5	<0.001
Surfactant therapy	16 (89)	13 (76)	0.330
Ureaplasma isolated	7 (39)	1 (6)	0.020
Mycoplasma isolated	5 (28)	1 (6)	0.086
PDA	8 (44)	4 (24)	0.193
Indomethacin*	16 (89)	10 (59)	0.034
Days MV†	42±13	7±2	<0.001
Days oxygen†	68±13	11±2	<0.001
Days hospital†	104±13	69±6	0.012
MV>28 days	9 (50)	0	<0.001
Supplemental O_2 at 36 weeks PCA	3 (18)	0	0.061
Antenatal corticosteroids	9 (50)	9 (53)	0.716
Postnatal corticosteroids	15 (83)	7 (41)	0.004
Age corticosteroids started, days	7±2	9±2	0.854
IVH	6 (33)	5 (29)	0.803
PVL	3 (18)	1 (6)	0.287

NOTE. Data are presented as mean±SEM or as numbers (percentages). Abbreviations: PDA, patent ductus arteriosus; PCA, postconceptional age; IVH, intraventricular hemorrhage; PVL, periventricular leukomalacia.

**Indomethacin was used prophylactically in most infants.*

†Wilcox rank sum test was used because data was not normally distributed.

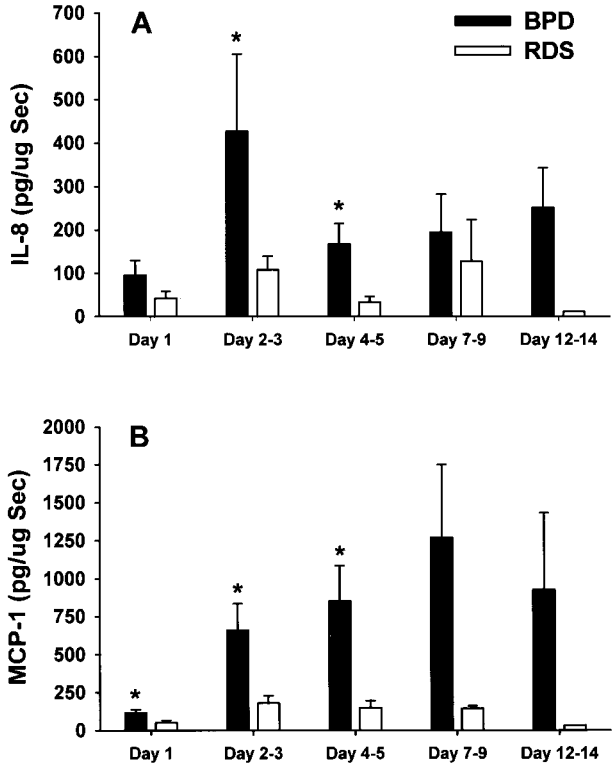

Figure 1. *TA concentrations of A) IL-8 and B) MCP-1 in infants who developed BPD (filled bars) and those who did not (open bars) during the first 14 days of life. All cytokines were normalized to secretory component. Concentrations are expressed as picograms of cytokine per microgram of secretory component±SEM. Differences between infants with BPD and those without were significant by ANOVA (*P*<0.001). *P<0.05 for individual time points. RDS, respiratory distress syndrome.*

Similarly, MCP-1 was also detected in all TA samples in both patient groups. TA concentrations of MCP-1 increased more gradually than those for IL-8, with maximal concentrations occurring between 7 and 9 days, especially in those infants who developed BPD (Figure 1B). TA concentrations of MCP-1 were greater in those infants who developed BPD during the first 2 weeks of life ($P<0.0001$, ANOVA). In addition, maximum MCP-1 concentrations were significantly higher in infants who subsequently developed BPD (1677±429 pg/μg/sc vs 194±44 pg/μg/sc; $P=0.003$). Furthermore, the magnitude of the inflammatory response correlated with the duration of ventilatory and oxygen support required. There was a significant correlation between maximum concentrations of IL-8 and MCP-1 and the duration of MV and oxygen therapy (Figure 2).

The role of birth weight, gestation, maximal cytokine concentrations, and Ureaplasma cultures in the development of BPD were examined by multiple logistic regression. This analysis showed that birth weight and maximum MCP-1 (but not maximum IL-8) concentrations correlated with the development of BPD in our cohort of infants. Because only three patients had persistent oxygen dependency at 36 weeks postconceptional age, analysis of the role of IL-8 and MCP-1 in the development of this more severe form of BPD could not be performed.

Differences in TA cytokine concentrations did not correlate to either birth weight or gestation. Additionally, postnatal corticosteroids did not significantly alter TA concentrations of either IL-8 (172±63 pg/μg/sc vs 269±212 pg/μg/sc; $P=0.657$) or MCP-1 (548±183 pg/μg/sc vs 213±56 pg/μg/sc; $P=0.126$) in the 15 pairs of samples available for analysis.

Maternal conditions did not have an effect on TA IL-8 or MCP-1 concentrations. TA concentrations of IL-8 and MCP-1 during the first 21 days of life were not different in infants whose mothers did or did not receive antenatal corticosteroids. Similarly, maximum MCP-1 (995±388 pg/μg/sc vs 960±360 pg/μg/sc; $P=0.946$) and IL-8

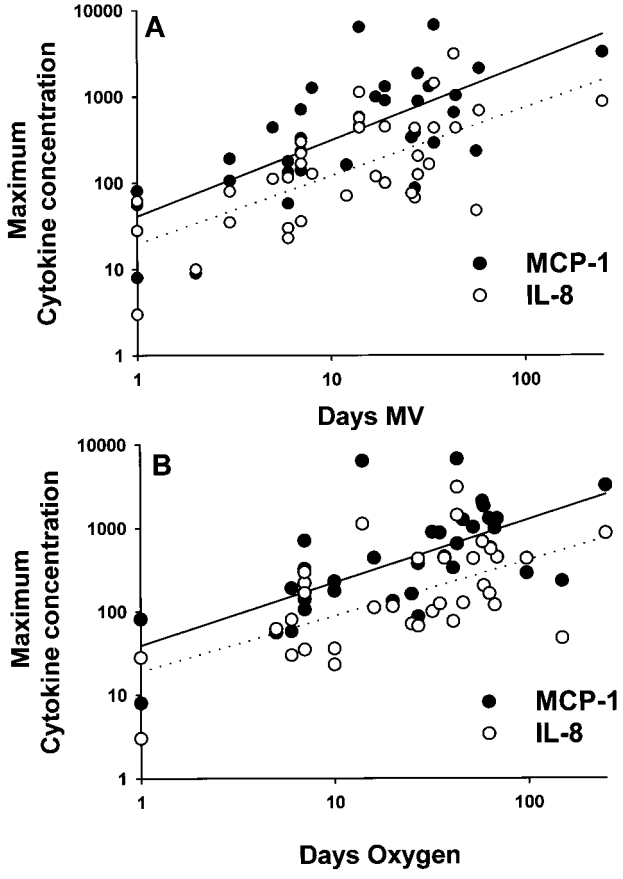

Figure 2. *Correlation of maximal TA concentrations of MCP-1 (filled circles) and IL-8 (open circles) with duration of A) MV and B) oxygen therapy in preterm infants. Data are expressed as the log-normalized cytokine concentration. Correlation coefficients for log MCP-1 vs MV,* $r^2=0.52$, $P<0.001$; *log IL-8 vs MV,* $r^2=0.47$, $P<0.001$; *MCP-1 vs days oxygen,* $r^2=0.47$, $P<0.001$; *log IL-8 vs days oxygen,* $r^2=0.39$, $P<0.001$.

(265±78 pg/μg/sc vs 452±173 pg/μg/sc; $P=0.323$) concentrations were not significantly different in infants whose mothers had received antenatal corticosteroids. The role of placental inflammation on TA cytokine concentrations and the subsequent development of BPD could not be assessed in this study, because too few placentas had histologic examination to allow a comparison.

Uu was isolated from TA cultures in eight (23%) study infants. Isolation of Uu from the TA was associated with an increased incidence of BPD, isolation of Mh, and a trend toward lower birth weight (Table 2). Seven of eight infants with Uu present in the TA were treated with erythromycin (40 mg/kg/d) for 10 days. Treatment of all these infants started in the first week of life. One patient, whose TA culture for Uu turned positive after the infant was extubated, was not treated. Two patients with tracheal

Table 2. Comparison between infants with and without isolation of Uu.

Characteristic	Ureaplasma (n=8)	No Ureaplasma (n=27)	*P*
Birth weight, g	779±50	929±42	0.076
Gestation, weeks	25.5±0.4	26.8±0.4	0.819
Surfactant therapy	6 (75)	24 (85)	0.502
Mycoplasma isolated	6 (75)	0 (0)	<0.001
PDA	3 (38)	9 (38)	0.827
Indomethacin*	7 (88)	19 (70)	0.330
BPD	6 (86)†	11 (44)	0.034
Days MV‡	29±7	24±10	0.073
Days oxygen‡	39±8	41±10	0.379
Days hospital‡	91±8	75±13	0.582
MV>28 days	3 (38)	6 (19)	0.246
Supplemental O_2 at 36 weeks PCA	0§	3 (11)	0.392
Antenatal corticosteroids	6 (75)	12 (44)	0.153
Postnatal corticosteroids	6 (75)	16 (61)	0.418
Age corticosteroids started, days	9±3	9±1	0.903
IVH	3 (38)	8 (30)	0.674
PVL	2 (25)	2 (7)	0.170

NOTE. Data are presented as mean±SEM or as numbers (percentages).
**Indomethacin was used prophylactically in most infants.*
†Only 7 infants survived to 28 days.
‡Wilcox rank sum test was used because data was not normally distributed.
§Only 6 infants survived to 36 weeks PCA.

Uu died of intractable respiratory failure at ages 14 and 43 days. There was only a single patient in our study that had a positive TA culture for bacteria (*Klebsiella pneumoniae*) at birth. In this patient, Uu was also isolated from the TA. This patient was not excluded from the study.

The TA concentrations of IL-8 in infants who had a positive culture for Uu increased during the course of their disease in a pattern similar to that of the entire group of patients who developed BPD (Figure 3A). TA concentrations of IL-8 were significantly increased in infants with tracheal cultures that were positive for Uu when compared with infants who did not have positive Uu tracheal cultures ($P=0.0032$, ANOVA). TA concentrations of MCP-1 were also significantly increased in infants with tracheal cultures that were positive for Uu when compared with infants who did not have positive Uu tracheal cultures ($P=0.002$, ANOVA) (Figure 3B). The pattern of the MCP-1 concentrations in Uu-positive infants paralleled that seen with the development of BPD. Because Mh was

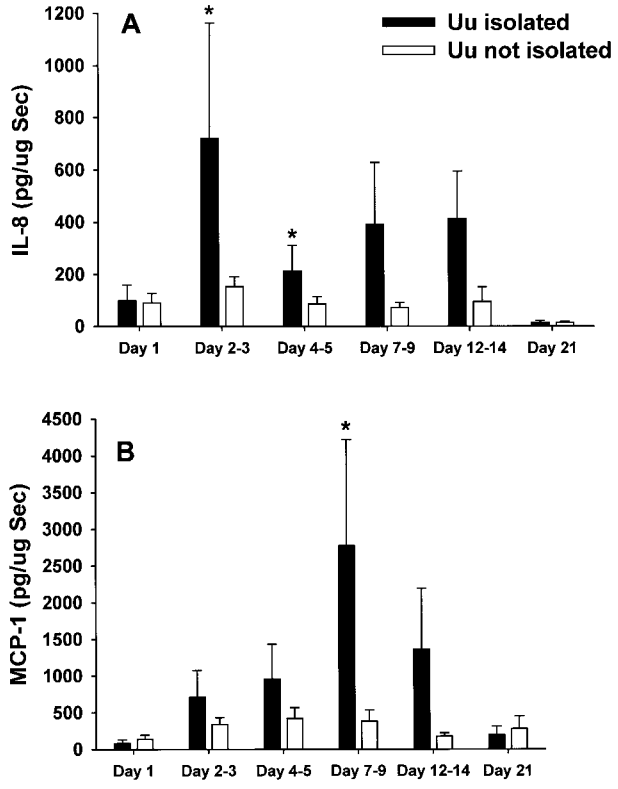

Figure 3. *TA concentrations of A) IL-8 and B) MCP-1 in infants with isolation of Uu from TA (filled bars) and those without (open bars) during the first 21 days of life. All cytokines were normalized to secretory component. Concentrations are expressed as picograms of cytokine per micrograms of secretory component±SEM. Differences between infants with TA isolation of Uu and those without were significant by ANOVA (P<0.001). *P<0.05 for individual time points.*

never recovered without the coisolation of Uu, an independent assessment of the role of Mh in BPD could not be ascertained.

DISCUSSION

Inflammation is one of the primary pathologic processes that precedes the development of BPD. Increased tracheal concentrations of inflammatory cytokines (IL-8, IL-1β, IL-6, TNFα, macrophage inflammatory protein-1α) have been reported in infants who develop the disease.[5–11] IL-8 is a major neutrophil chemoattractant and activator. The concentrations of this chemokine in TA have been shown to be elevated early in the course of BPD, a time when neutrophil influx is highest.[1,11,12] Our findings, establishing a correlation between IL-8 and the development of BPD, are similar to the results reported by others.[7,11]

MCP-1, a member of the CC chemokine family, induces the migration and activation of mononuclear cells.[12] MCP-1 has been implicated in the pathogenesis of lung fibrosis in human disease and bleomycin-induced pulmonary fibrosis in animal models.[13–17] In addition, MCP-1 concentrations correlate with lung injury during the late phase of the adult respiratory distress syndrome.[27] The correlation of increased MCP-1 with BPD in our study suggests a role for this chemokine in the later phase of this disease. The kinetics and duration of elevated MCP-1 concentrations may coincide better with the early stages of fibrosis than does IL-8, which coincides more closely with the initial inflammatory response. This observation suggests that the increase in lung MCP-1 and the resultant monocyte recruitment and activation may be of greater importance to the development of the fibrotic component of BPD than the early increases in IL-8. This hypothesis is further supported by multiple logistic regression analysis, in which maximum MCP-1, but not maximum IL-8, concentrations were predictive of BPD.

We speculate that MCP-1 may play a role in the fibrosis that occurs in BPD. Monocytes/macrophages recruited to the lung, and subsequently activated by MCP-1, may release other growth factors that stimulate fibroblast proliferation, differentiation, and collagen production.[28,29] Additionally, MCP-1 could indirectly promote fibroblast collagen production by stimulating fibroblasts to produce transforming growth factor β_1.[30]

The role of Uu colonization or infection in the development of chronic lung disease is unclear. Most, but not all studies, suggest that isolation of Uu from TA is associated with an increased incidence of chronic lung disease in very low birth weight infants.[19–21] The frequency of Uu isolation in our infants and the increased incidence of BPD is in agreement with other reports.[19–21] Repeat cultures were not performed on most infants in our study. Thus, we may have underestimated the incidence of these infections in our infants.

Our study was not designed to assess the efficacy of treatment strategies for Uu. Treatment of our infants with erythromycin did not seem to reduce the incidence of oxygen dependency at 28 days. This may be a result of specific treatment starting after TA cultures were positive. Treatment, after the cultures return positive, may occur after the inflammatory response is well established in the lung and, thus, may be less effective. Some of the observed differences in TA concentrations of IL-8 and MCP-1 between infants who develop BPD and those who do not may be related to infection by Uu.

Concentrations of IL-8 and MCP-1 were greater in the Uu-positive group than in infants without Uu isolation and the overall BPD group, suggesting that a significant pro-

portion of the cytokine response in the BPD group may be a result of Uu infection. Increased TA concentrations of IL-8, IL-1β, and TNFα have been reported in infants with respiratory tract isolation of Uu.[22,23] Similarly, we found increased concentrations of IL-8 and MCP-1 in tracheal fluid from infants with Uu isolates. In contrast to our study, Lyon et al[31] reported no increase in TA IL-8 concentrations in infants with isolation of Uu. Differences in results may reflect differences in patient populations, sampling techniques, and incidence of and/or severity of Ureaplasmal infections.

Our findings lend support to the contention that Uu plays a pathologic role in the development of BPD in some infants. These clinical findings are in agreement with the experimental evidence that Uu can induce cytokine production in pulmonary fibroblasts, macrophages, and respiratory epithelial cells.[32–36] The frequent coisolation of Mh in our patient population makes independent assessment of the relative roles of Uu and Mh difficult. Mh is capable of inducing cytokines in macrophages.[33,34] Furthermore, we have demonstrated that Mh can induce IL-8, MCP-1, and ENA-78 in cultured respiratory epithelial cells.[35,36] Thus, Mh infection of the lung or airways may also contribute to the cytokine responses we observed.

In summary, we have shown that TA concentrations of IL-8 and MCP-1 are significantly increased in infants who subsequently developed BPD. Concentrations of IL-8 and MCP-1 correlated well with duration of oxygen therapy and MV. IL-8 concentrations increased early in the course of the disease, whereas MCP-1 concentrations increased later. Increased TA concentrations of IL-8 and MCP-1 found in infants who developed BPD were associated with the isolation of Uu, further supporting a role for Uu as a pathogen in the perinatal period. Finally, MCP-1 concentrations predicted the development of BPD better than IL-8, suggesting a predominant role of this chemokine in the development of this disease.

ACKNOWLEDGMENTS

We thank the nurses and respiratory therapists in the neonatal intensive care unit who helped with specimen collection.

REFERENCES

1. Merritt TA, Stuard ID, Puccia J, Wood B, Edwards DK, Finkelstein J, Shapiro DL. Newborn tracheal aspirate cytology: Classification during respiratory distress syndrome and bronchopulmonary dysplasia. J Pediatr 1981;98:949–956.

2. Rindfleisch MS, Hasday JD, Taciak V, Broderick K, Viscardi RM. Potential role of interleukin-1 in the development of bronchopulmonary dysplasia. J Interferon Cytokine Res 1996;16:365–373.

3. Ogden BE, Murphy S, Saunders GC, Johnson JD. Lung lavage of newborns with respiratory distress syndrome. Prolonged neutrophil influx is associated with bronchopulmonary dysplasia. Chest 1983;83: 31S–33S.

4. Groneck P, Götze-Speer B, Oppermann M, Eiffert H, Speer CP. Association of pulmonary inflammation and increased microvascular permeability during the development of bronchopulmonary dysplasia: A sequential analysis of inflammatory mediators in respiratory fluids of high-risk preterm neonates. Pediatrics 1994;93:712–718.

5. Jonsson B, Tullus K, Brauner A, Lu Y, Noack G. Early increase of TNF alpha and IL-6 in tracheobronchial aspirate fluid indicator of subsequent chronic lung disease in preterm infants. Arch Dis Child Fetal Neonatal Ed 1997;77:198–201.

6. Murch SH, Costeloe K, Klein NJ, MacDonald TT. Early production of macrophage inflammatory protein-1 alpha occurs in respiratory distress syndrome and is associated with poor outcome. Pediatr Res 1996;40:490–497.

7. Tullus K, Noack GW, Burman LG, Nilsson R, Wretlind B, Brauner A. Elevated cytokine levels in tracheobronchial aspirate fluids from ventilator treated neonates with bronchopulmonary dysplasia. Eur J Pediatr 1996;155:112–116.

8. Kotecha S, Wilson L, Wangoo A, Silverman M, Shaw RJ. Increase in interleukin (IL)-1 beta and IL-6 in bronchoalveolar lavage fluid obtained from infants with chronic lung disease of prematurity. Pediatr Res 1996;40:250–256.

9. Kotecha S, Chan B, Azam N, Silverman M, Shaw RJ. Increase in interleukin-8 and soluble intercellular adhesion molecule-1 in bronchoalveolar lavage fluid from premature infants who develop chronic lung disease. Arch Dis Child Fetal Neonatal Ed 1995;72:F90–F96.

10. Watterberg KL, Demers LM, Scott SM, Murphy S. Chorioamnionitis and early lung inflammation in infants in whom bronchopulmonary dysplasia develops. Pediatrics 1996;97:210–215.

11. Munshi UK, Niu JO, Siddiq MM, Parton LA. Elevation of interleukin-8 and interleukin-6 precedes the influx of neutrophils in tracheal aspirates from preterm infants who develop bronchopulmonary dysplasia. Pediatr Pulmonol 1997;24:331–336.

12. Mukaida N, Harada A, Matsushima K. Interleukin-8 (IL-8) and monocyte chemotactic and activating factor (MCAF/MCP-1), chemokines essentially involved in inflammatory and immune reactions. Cytokine Growth Factor Rev 1998;9:9–23.

13. Zhang K, Phan SH. Cytokines and pulmonary fibrosis. Biol Signals 1996;5:232–239.

14. Smith RE, Strieter RM. Zhang K, Phan SH, Standiford TJ, Lukacs NW, Kunkel SL. A role for C-C chemokines in fibrotic lung disease. J Leukoc Biol 1995;57:782–787.

15. Vaillant P, Menard O, Vignaud JM, Martinet N, Martinet Y. The role of cytokines in human lung fibrosis. Monaldi Arch Chest Dis 1996;51:145–152.

16. Smith RE, Strieter RM, Phan SH, Kunkel SL. C-C chemokines. Novel mediators of the profibrotic inflammatory response to bleomycin challenge. Am J Respir Cell Mol Biol 1996;15:693–702.

17. Car BD, Meloni F, Luisetti M, Semenzato G, Gialdroni-Grassi G, Walz A. Elevated IL-8 and MCP-1 in the bronchoalveolar lavage fluid of patients with idiopathic pulmonary fibrosis and pulmonary sarcoidosis. Am J Respir Crit Care Med 1994;149:655–659.

18. Standiford TJ, Huffnagle GB. Cytokines in host defense against pneumonia. J Investig Med 1997;45:335–345.

19. Cassell GH, Waites KB, Crouse DT, Rudd PT, Canupp KC, Stagno S, Cutter GR. Association of *Ureaplasma urealyticum* infection of the lower respiratory tract with chronic lung disease and death in very-low-birth-weight infants. Lancet 1988;2:240–245.

20. Wang EE, Frayha H, Watts J, Hammerberg O, Chernesky MA, Mahony JB, Cassell GH. Role of *Ureaplasma urealyticum* and other pathogens in the development of chronic lung disease of prematurity. Pediatr Infect Dis J 1998;7:547–551.

21. Wang EE, Ohlsson A, Kellner JD. Association of *Ureaplasma urealyticum* colonization with chronic lung disease of prematurity: Results of a metaanalysis. J Pediatr 1995;127:640–644.

22. Groneck P, Goetze-Speer B, Speer CP. Inflammatory bronchopulmonary response of preterm infants with microbial colonization of the airways at birth. Arch Dis Child Fetal Neonatal Ed 1996;74:F51–F55.

23. Patterson AM, Taciak V, Lovchik J, Fox RE, Campbell AB, Viscardi RM. *Ureaplasma urealyticum* respiratory tract colonization is associated with an increase in interleukin 1-beta and tumor necrosis factor alpha relative to interleukin 6 in tracheal aspirates of preterm infants. Pediatr Infect Dis J 1998;17:321–328.

24. Palta M, Sadek M, Barnet JH, Evans M, Weinstein MR, McGuinness G, Peters ME, Gabbert D, Fryback D, Farrell P. Evaluation of criteria for chronic lung disease in surviving very low birth weight infants. J Pediatr 1998;132:57–63.

25. Isenberg HD, ed. Clinical Microbiology Procedures Handbook. Washington, DC: American Society for Microbiology; 1994.

26. Watts CL, Bruce MC. Comparison of secretory component for immunoglobulin A with albumin as reference proteins in tracheal aspirate from preterm infants. J Pediatr1995;127:113–122.

27. Goodman RB, Strieter RM, Martin DP, Steinberg KP, Milberg JA, Maunder RJ, Kunkel SL, Walz A, Hudson LD, Martin TR. Inflammatory cytokines in patients with persistence of the acute respiratory distress syndrome. Am J Respir Crit Care Med 1996;154:602–611.

28. Temelkovski J, Kumar RK, Maronese SE. Enhanced production of an EGF-like growth factor by parenchymal macrophages following bleomycin-induced pulmonary injury. Exp Lung Res 1997;23:377–391.

29. Maeda A, Hiyama K, Yamakido H, Ishioka S, Yamakido M. Increased expression of platelet-derived growth factor A and insulin-like growth factor-I in BAL cells during the development of bleomycin-induced pulmonary fibrosis in mice. Chest 1996;109:780–786.

30. Gharaee-Kermani M, Denholm EM, Phan SH. Costimulation of fibroblast collagen and transforming growth factor beta1 gene expression by monocyte chemoattractant protein-1 via specific receptors. J Biol Chem 1996;271:17779–17784.

31. Lyon AJ, McColm J, Middlemist L, Fergusson S, McIntosh N, Ross PW. Randomised trial of erythromycin on the development of chronic lung disease in preterm infants. Arch Dis Child Fetal Neonatal Ed 1998;78:F10–F14.

32. Stancombe BB, Walsh WF, Derdak S, Dixon P, Hensley D. Induction of human neonatal pulmonary fibroblast cytokines by hyperoxia and *Ureaplasma urealyticum*. Clin Infect Dis 1993;17(suppl 1): S154–S157.

33. Crouse DT, English BK, Livingston L, Meals EA. Genital mycoplasmas stimulate tumor necrosis factor-alpha and inducible nitric oxide synthase production from a murine macrophage cell line. Pediatr Res 1998;4:785–790.

34. Talati AJ, Crouse DT, English K, Newman C, Livingston L, Meals E. Exogenous bovine surfactant suppresses tumor necrosis factor-alpha release by murine macrophages stimulated by genital mycoplasmas. J Infect Dis 1998;178:1122–1125.

35. Baier RJ, Kruger TE. Induction of monocyte chemoattractant protein-1 by *Mycoplasma hominis* in respiratory epithelial cells. J Investig Med 2000;48:457–464.

36. Kruger T, Baier J. Induction of neutrophil chemoattractant cytokines by *Mycoplasma hominis* in alveolar type II cells. Infect Immun 1997;65:5131–5136.