INTERLEUKIN-8 IN BRONCHOALVEOLAR LAVAGE FLUID OF PREMATURE INFANTS AT RISK OF CHRONIC LUNG DISEASE

Bai-Horng Su, Hsiao-Yu Chiu, Tsung-Wen Lin, and Hung-Chih Lin

Background and Purpose: Persistence of neutrophils in the tracheal fluid of premature infants is associated with chronic lung disease (CLD). Interleukin-8 (IL-8) is a potent neutrophil chemoattractant. This study investigated whether IL-8 is increased in the bronchoalveolar lavage fluid of premature infants with different types of CLD.

Methods: Forty two very low birth weight infants who required mechanical ventilation were recruited. Twenty eight of these infants developed CLD and 14 infants recovered without developing CLD. Four additional infants receiving mechanical ventilation for non-respiratory reasons were also enrolled as controls. CLD was defined as requirement for supplemental oxygen at 28 days of age and chest radiograph showing characteristic appearance. CLD was further classified into 3 subtypes: bronchopulmonary dysplasia (BPD), Wilson-Mikity syndrome (WMS) and chronic pulmonary insufficiency of prematurity (CPIP).

Results: IL-8 in bronchoalveolar lavage fluid was significantly increased in the CLD group by 8 days of age compared to those who did not develop CLD (p < 0.05). For infants without CLD, IL-8 increased from 963 pg/mL on day 1 after delivery to 1463 pg/mL on day 4, and decreased to 1000 pg/mL on day 8. For infants with BPD, IL-8 increased from 925 pg/mL on day 1 after delivery to 2650 pg/mL on day 8, and then gradually decreased to 1500 pg/mL on day 28. Infants with WMS had significantly higher IL-8 from the first day after delivery (4567 pg/mL) than infants with BPD or CPIP and this difference persisted to age 28 days (2475 pg/mL).

Conclusions: Persistent inflammation could be a major contributory factor in the development of CLD. The different patterns of response to inflammation in different types of CLD may have implications for the design of appropriate strategies to prevent and treat CLD.

Key words: Bronchopulmonary dysplasia; Chronic diseases; Infant, very low birth weight; Interleukin-8; Lung diseases

Chronic lung disease (CLD) is an important complication of extreme prematurity. CLD of premature infants has a multifactorial etiology. Oxygen toxicity, mechanical injury (barotrauma and volutrauma) as well as prenatal and postnatal infections may contribute to pulmonary injury in the immature lung of premature infants.

In infants with respiratory distress syndrome (RDS) who require mechanical ventilation, there are few neutrophils in tracheal fluid at birth but numbers increase to maximum by 1-2 days of age and decrease to baseline values by the end of the first week of life. However, in infants who progress to CLD, neutrophils in tracheal fluid may persist for several weeks. Interleukin-8 (IL-8), a marker of inflammation and a potent neutrophil chemoattractant, promotes neutrophil degranulation, and induces a weak but rapid respiratory burst in neutrophils. IL-8 is increased in acute lung injury, including adult RDS and idiopathic pulmonary fibrosis. Furthermore, hyperoxia, to which ventilated premature infants are frequently exposed, has been shown to result in a 4-fold increase in IL-8 gene expression in monocytes when compared with normoxia.

We have reported that the incidence of CLD in extremely low birth weight infants that survived beyond 28 days was 50.7% and the severity of CLD may vary greatly from bronchopulmonary dysplasia (BPD) or Wilson-Mikity syndrome (WMS) with severe respiratory morbidity and mortality to chronic pulmonary insufficiency of prematurity (CPIP) with no residual problems. This implies that different types of CLD may differ with respect to pathogenesis.

The persistence of neutrophils in the tracheal fluid of premature infants is associated with CLD, and IL-8 is a potent neutrophil chemoattractant. This study investigated whether IL-8 is increased in the bronchoalveolar lavage fluid obtained from...
premature infants with different types of CLD and infants who did not develop CLD.

**Methods**

**Patients**

Infants with birth weight less than 1500 g who required mechanical ventilation and were admitted to the neonatal intensive care unit at China Medical University Hospital were recruited for this study. CLD was defined as a supplemental oxygen requirement at 28 days of age, with symptoms of persistent respiratory distress and chest radiograph showing characteristic appearance. CLD was further classified according to previous reports into 3 subtypes: BPD, WMS and CPIP. In addition to the common findings of CLD described above, infants with BPD had a history of RDS, infants with WMS did not have RDS but had early appearance of bubbly lung on chest X-ray within first week of life, and infants with CPIP had or did not have RDS in early life but had a hazy appearance on chest X-ray at 28 days of age at the assessment of CLD was performed. Four infants receiving mechanical ventilation for non-respiratory reasons (including asphyxia and surgery; no RDS, not inflammatory) and not dependent on supplementary oxygen by 28 days of age (no CLD) were enrolled as control. Informed consent was obtained from the parents and the study was approved by the institutional review board of the hospital.

**Bronchoalveolar lavage**

Each intubated infant received tracheal suction every 4 to 6 hours. The first lavage was performed 4 to 6 hours after surfactant treatment (Survanta; Abbott Laboratories). If infants did not receive surfactant, the first lavage was performed at the first tracheal suction during the first day. Bronchoalveolar lavage was performed as follows. With the infant lying supine, and head turned to the right, 1.5 mL/kg of saline was instilled into the endotracheal tube, which was then reconnected to the ventilator for 30 seconds. The fluid was regained with a 6 FG suction catheter attached to a mucus extractor with a control suction connector, and suction pressure between 15 and 20 cm H₂O. Heart rate, blood pressure and pulse oximetry were monitored throughout the procedure. Infants were lavaged twice weekly for 4 weeks or until extubation, depending on which occurred earlier.

**Interleukin-8**

Bronchoalveolar lavage fluid was stored at temperature of –80°C until measurement of IL-8 was performed using a commercially available enzyme-linked immunosorbant assay (ELISA) kit (R & D Quantikine Human IL-8 D8000, Minneapolis, Minnesota, USA). Each sample, in each ELISA, was measured in duplicate.

**Statistics**

Data on IL-8 are presented as mean values. Categorical variables and prevalence data were analyzed using chi-squared test. Other variables were analyzed using Wilcoxon rank sum test. A *p* value of < 0.05 was considered statistically significant.

**Results**

Forty two very low birth weight infants who required mechanical ventilation were recruited for the study. Among them, 28 infants developed CLD and 14 recovered without developing CLD. The patient data are summarized in the Table. Infants with CLD had significantly lower gestational weeks and lower birth weight than infants without CLD and infants of the control group. Eighteen of 28 mothers in the CLD group and 10 of 14 mothers in the no CLD group received prenatal steroid. There was no significant difference between the 2 groups in the prevalence

**Table. Clinical characteristics of the study subjects.**

<table>
<thead>
<tr>
<th>Characteristic</th>
<th>CLD (n = 28)</th>
<th>No CLD (n = 14)</th>
<th>Controls (n = 4)</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>BPD (n = 14)</td>
<td>WMS (n = 4)</td>
<td>CPIP (n = 10)</td>
</tr>
<tr>
<td>Gestational age (weeks)</td>
<td>26.4 ± 2.2</td>
<td>25.7 ± 1.2</td>
<td>25.8 ± 1.6</td>
</tr>
<tr>
<td>Birth weight (g)</td>
<td>905 ± 255</td>
<td>730 ± 112</td>
<td>832 ± 124</td>
</tr>
<tr>
<td>RDS/surfactant (v/n)</td>
<td>22/21</td>
<td>14/14</td>
<td>0/0</td>
</tr>
<tr>
<td>Prenatal steroid</td>
<td>18 (66.7%)</td>
<td>9 (64.3%)</td>
<td>3 (75%)</td>
</tr>
<tr>
<td>PROM</td>
<td>8 (28.6%)</td>
<td>3 (21.4%)</td>
<td>4 (100%)</td>
</tr>
</tbody>
</table>

*Values are mean ± SD or number (%). v/n indicates number of infants with RDS/number of infants receiving surfactant.
*p* < 0.05, †*p* < 0.01 for CLD group vs no CLD or control groups.
‡*p* < 0.01 for BPD and WMS groups vs CPIP group.
.§*p* < 0.01 for WMS group vs BPD and CPIP groups.

CLD = chronic lung disease; BPD = bronchopulmonary dysplasia; WMS = Wilson-Mikity syndrome; CPIP = chronic pulmonary insufficiency of prematurity; RDS = respiratory distress syndrome; PROM = prolonged rupture of membrane.
of prenatal steroid use, RDS, surfactant supplement and maternal premature rupture of membrane. Comparison of different types of CLD revealed that infants with BPD or WMS had significantly lower gestational weeks and lower birth weight than infants with CPIP. There was no significant difference in maternal prenatal steroid use among different types of CLD with 9 of 14 infants in the BPD group, 3 of 4 in the WMS group and 6 of 10 in the CPIP group.

IL-8 in the bronchoalveolar lavage fluid of controls decreased gradually from 952 pg/mL initially to 612 pg/mL by 8 days of age and around 500-600 pg/mL thereafter. Among infants without CLD, IL-8 increased from 963 pg/mL on day 1 after delivery to 1463 pg/mL on day 4, and decreased to 1000 pg/mL on day 8. In the CLD group, IL-8 increased gradually and was significantly higher as early as within 8 days of age as compared with the controls or no CLD group (Fig. 1).

In infants with BPD, IL-8 increased from 925 pg/mL on day 1 after delivery to 2650 pg/mL on day 8, and then decreased gradually to 1500 pg/mL on day 28. Infants with CPIP also had an initial increase in IL-8 until day 8 and then decreased gradually to near the level of IL-8 of infants without CLD. Infants with WMS had significantly higher IL-8 since the first day after delivery (4567 pg/mL) than infants with BPD or CPIP, and this difference persisted to 28 days (2475 pg/mL) (Fig. 2).

**Discussion**

CLD is much more common in infants with immature lungs, especially those infants with gestational age less than 28 weeks and birth weight less than 1000 g. This study found that infants with CLD had significantly lower gestational age and lower birth weight than those without CLD and controls.

In bronchoalveolar lavage fluid, IL-8, a marker of inflammation, was increased in infants who developed CLD as compared with infants without CLD or controls (Fig. 1). The increase in IL-8 appeared as early as 8 days of age in infants who subsequently developed CLD and reached peak concentration by 8 days of age. This finding suggests that IL-8 is a contributor to lung inflammation as reflected by the rapid decrease to low concentration by 8 days of age in infants without CLD and the low concentration of IL-8 detected in lavage fluid obtained from controls, and suggests that measurement of IL-8 in bronchoalveolar lavage fluid at 8 days of age may predict the subsequent development of CLD.

Comparison of infants with different types of CLD revealed that those with BPD or WMS had significantly higher IL-8 in bronchoalveolar lavage fluid than infants with CPIP during the study course. Infants with WMS had significantly higher IL-8 in bronchoalveolar lavage fluid since the first day of age than infants with BPD or CPIP and this difference persisted to 28 days of age. IL-8 in infants with CPIP decreased gradually from its peak value at day 8 to approach the level of IL-8 of infants without CLD (Fig. 2). Because there was no significant difference in the prevalence of maternal prenatal steroid use, the difference of IL-8 in bronchoalveolar lavage fluid among these 3 types of CLD could not be attributed to prenatal steroid administration. Our previous study found that
infants with CPIP have less severe clinical condition than infants with BPD or with WMS,1 and the gradual decrease of IL-8 of infants with CPIP in this study may reflect this difference.

In this study, infants with WMS had a significantly higher incidence of maternal premature rupture of membranes compared with infants with BPD or with CPIP. A possible explanation for the significantly higher IL-8 in bronchoalveolar lavage fluid in these infants is the prolonged inflammation of the chorionic plate, which has been termed subacute chorioamnionitis and may provide a trigger for early pulmonary inflammation.20–22

IL-8 was increased in other forms of acute lung injury such as acute RDS (ARDS).23 Bronchoalveolar lavage fluid from patients with ARDS also contains elevated levels of IL-8. The importance of IL-8 in pulmonary inflammation was demonstrated by the presence of anti-IL-8 autoantibody in the bronchoalveolar lavage fluid from ARDS patients and BPD infants.23,24

In summary, this study revealed that persistent inflammation could be a major contributory factor in the development of CLD. Measurement of IL-8 in bronchoalveolar lavage fluid at 8 days of age might help identify infants who are at increased risk of subsequent development of CLD and who therefore may benefit from early treatment with anti-inflammatory agents such as anti-IL-8.25,26 The different patterns of time course of IL-8 in bronchoalveolar lavage fluid in different types of CLD may be associated with different patterns of response to inflammation. Such information may be helpful for designing appropriate strategies to prevent CLD. IL-8 measurement at 8 days of age in lavage fluid may be used to assess the response to therapy during the first few days after delivery.

References

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