Lactoferrin and lysozyme deficiency in airway secretions: Association with the development of bronchopulmonary dysplasia

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To test whether the presence of airway inflammatory markers differentiated babies with hyaline membrane disease (HMD) who recovered (n = 18) from those in whom bronchopulmonary dysplasia (BPD) developed (n = 18), tracheal aspirate samples from 36 newborn infants with HMD who underwent intubation were collected during days 1 to 28 of life and analyzed for the mucosal antimicrobial proteins lactoferrin and lysozyme. For babies with HMD in whom BPD developed, lactoferrin concentrations were decreased during the first 4 days of life (7 ± 3, 14 ± 3, 18 ± 3, and 18 ± 3 μg/ml, respectively) in comparison with those in babies with HMD who recovered (23 ± 8, 29 ± 6, 41 ± 9, and 81 ± 19 μg/ml); group differences reached statistical significance on days 3 and 4 (_p_ <0.05). Lysozyme levels in the secretions of babies with BPD were also lower on day 3 (31 ± 5 μg/ml) than in those of babies who recovered (54 ± 7.5 μg/ml). For babies with BPD whose endotracheal tube remained in place beyond day 4, lysozyme levels on days 5 to 12 were significantly lower for those classified as having severe BPD than for those with mild to moderate BPD. Because lysozyme and lactoferrin are products of serous cells found in submucous glands, it seems possible that the relative immaturity of submucous glands may influence the development of BPD. (J PEDIATR 1992;121:262-70)

Airway inflammation has recently received attention as an important contributor to the lung injury associated with both hyaline membrane disease and bronchopulmonary dysplasia. Significant numbers of neutrophils and macrophages are found in pulmonary fluid obtained from infants with HMD and BPD.[1,2] The presence of inflammatory cells suggests the possibility that they may participate in the development of lung disease, because several neutrophil products are potentially toxic to lung tissue.[3] Moreover, infants in whom BPD develops may be more susceptible to damage

from inflammatory cell products because of reduced antiprotease activity.[1]

Both specific (secretory IgA) and nonspecific (lysozyme, lactoferrin) molecules participating in mucosal host defense are secreted by serous cells of the submucosal glands.[4] These molecules represent the major proteins in respiratory

BPD	Bronchopulmonary dysplasia
HMD	Hyaline membrane disease
PBSS	Phosphate-buffered saline solution

epithelial lining fluid, and it is likely that deficiency of these molecules might predispose infants to altered host defense or response to injury. The two potential sources of lactoferrin and lysozyme are the serous cells of the submucous glands[5] and, to a lesser extent, the specific granules of neu-

trophils.[6] Lactoferrin concentrations can reflect the presence of serous secretions and have also been used as an indirect measurement of neutrophil-enriched inflammation.[7]

In this study, tracheal aspirates from newborn infants with HMD were collected and analyzed for lactoferrin and lysozyme. We hypothesized that the levels of neutrophil-derived lactoferrin and lysozyme would be increased in the airway fluid from babies "predisposed" to the development of BPD, and that these measurements might reveal a recognizable profile of tracheal aspirate composition that could be used to predict accurately greater risk for the development of BPD.

METHODS

Materials. Sterile specimen traps (Sherwood Medical Co., St. Louis, Mo.) were used to collect the tracheal aspirate. The materials purchased for this study included human serum albumin, o-phenylenediamine dihydrochloride, human lactoferrin, lysozyme standard, and *Micrococcus lysodeikticus* (Sigma Chemical Co., St Louis, Mo.); 96-well polypropylene microtiter plates (Becton Dickinson Labware, Lincoln Park, N.J.); rabbit anti-human lactoferrin (Dako Corp., Santa Barbara, Calif.); goat anti-human serum albumin–horse radish peroxidase conjugate and rabbit anti-human lactoferrin–horseradish peroxidase conjugate (Organon Teknika Corp., Durham, N.C.); normal goat serum (Gibco, Grand Island, N.Y.); polysorbate 80 (Tween 80; Fisher Scientific Co., Pittsburgh, Pa.); and Diff-Quik (Baxter Healthcare Corp., Scientific Division, McGaw Park, Ill.).

Subjects. All subjects were newborn infants who were transferred in the first 24 hours of life to the intensive care nursery at Children's National Medical Center, Washington, D.C., between Sept. 27, 1985, and July 24, 1986. Subjects were eligible to enter into the study if they had endotracheal tubes in place and were given supplemental oxygen for the treatment of HMD. The diagnosis of HMD was made clinically for premature infants (≤36 weeks of gestational age) with respiratory distress who had grunting, retractions, and nasal flaring; a chest radiograph consistent with HMD (typically, diffuse pulmonary "whiteout" or atelectasis with a reticulogranular pattern and air bronchograms); and the absence of other known causes of respiratory distress such as pneumonia, sepsis, neurologic disease, or pneumothorax. All infants had cultures and were treated for bacterial sepsis (ampicillin and gentamicin) until culture results were available. Tracheal aspirates were obtained within a few hours of intubation and sent for routine bacterial culture and Gram stain. Babies were excluded from the study if initial routine bacterial cultures of blood, spinal fluid, urine, or tracheal aspirate had bacterial growth. Viral cultures were not obtained. Testing for *Ureaplasma*

Table I. BPD score

	Points
Duration of oxygen	
<6 wk	1
6 wk to 3 mo	2
>3 mo	3
Duration of mechanical ventilation	
≤3 wk	1
>3 wk	2
Use of steroids for BPD	1
Death caused by BPD	3

Score: mild to moderate BPD, 1 to 4 points; severe BPD, 5 to 10 points.

was not done. For all babies, Gram stain of the initial tracheal aspirate showed no organisms and no or rare polymorphonuclear cells. All babies had negative urine and/or blood latex agglutination test results for group B streptococci. No mother was pretreated with antibiotics at the time of delivery. Subsequent bacterial cultures were obtained only if clinically indicated. In no case was the endotracheal tube used to administer medications or exogenous surfactant. In an effort to form a more homogeneous sample, babies who were not of an appropriate birth weight for their gestational age, or who had cyanotic heart disease or other congenital defects, were excluded.

Babies who recovered uneventfully from HMD (HMD group) were compared with babies with HMD in whom BPD subsequently developed (BPD group). The diagnosis of BPD was made if (1) supplemental oxygen was required for 3 or more weeks to prevent hypoxia from lung disease; (2) tachypnea and retractions persisted for more than 3 weeks; and (3) persistent changes on chest radiographs (obtained for clinical indications) were consistent with chronic lung disease as described by Edwards[8] (score unspecified). A period of 3 weeks or more of supplemental oxygen was chosen as the criterion for BPD to allow both mild and severe cases to be included in the study. Chest radiographs were interpreted by a pediatric radiologist and confirmed by a designated pediatric radiologist who was unaware of the patient's status in the study. All patients in the HMD group initially had chest radiographs typical of HMD. All babies with HMD had normal chest radiographs by 2 weeks of age and had no signs of BPD.

BPD score. To quantitate the degree of clinical severity of BPD, we devised a BPD score before the start of the study. As shown in Table I, points were given for the duration of oxygen requirement for lung disease, for the duration of mechanical ventilation for lung disease, for the requirement of systemic steroid therapy (dexamethasone) for BPD, and for death from BPD. The number of days of intubation or oxygen supplementation, or both, for apnea alone was specifically not included in the scores. A maxi-

mum score of 10 could be obtained. Scores of 1 to 4 were classified as indicating mild to moderate BPD. Scores of 5 to 10 were classified as indicating severe BPD. Patients were labeled as having the complication of bronchospasm if repeated episodes of wheezing developed (often with carbon dioxide retention), and if they required the long-term use of aminophylline or the daily use of bronchodilator aerosols, or both, to improve respiratory status. The use of dexamethasone was scored only if it was used by the attending neonatologist to improve the respiratory status of a ventilator-dependent baby with chronic lung disease.

Patient data collection. Information obtained from patient records included birth weight, gestational age, gender, race, maternal steroid administration, feeding history, and ventilator settings and arterial blood gas values at each sample collection time. In addition, medications and significant events in the clinical course were monitored, including the development of complications such as infection, pneumothorax, patent ductus arteriosus, and repeated episodes of bronchospasm.

Sample collection. Tracheal aspirates were collected once daily during the intubation period, days 1 to 28 of life, or until extubation if this occurred before 28 days. Samples were never pooled. The procedure for endotracheal tube aspiration was standardized for this study but was similar to the routine procedure used in our nursery for the care of all infants during intubation. When clinically indicated for pulmonary toilet, tracheobronchial aspiration was performed by instilling a total of 2 ml of 0.9% saline solution in 0.5 ml aliquots into the endotracheal tube; this is the usual volume used in our nursery for endotracheal tube suctioning and the regimen was deemed safe for even the smallest premature infant. After each instillation, the baby underwent hand ventilation to disperse the saline solution, and then the trachea was suctioned via a catheter slightly beyond the distal end of the endotracheal tube. Secretions were aspirated into a collection trap. The suction catheter was rinsed with 1 ml of saline solution into the collection trap to remove secretions adherent to the inside of the suction catheter.

Samples were immediately placed on ice and processed as quickly as possible. The volume of the aspirate was measured. The secretions were then centrifuged at 1500 g at 4° C for 10 minutes. Supernatants and pellets have been examined after this process and are free of visible cell fragments. The supernatant was saved and frozen at −70° C until assayed.

Cell counts. Some tracheal aspirates were collected for analysis of polymorphonuclear cells. The cell pellet was resuspended in isotonic saline solution and the cells were stained with 2% trypan blue. Cell counts were obtained with a hemocytometer. Differential cell counts were performed by smearing cells on slides and staining with Diff-Quik.

Albumin analysis. Albumin was measured by a specific, competitive enzyme-linked immunosorbent assay.[9] Measurements were made in triplicate and expressed as average micrograms per milliliter.

Lactoferrin analysis. Lactoferrin was measured by enzyme-linked immunosorbent assay using a double-antibody sandwich technique. A modification of the method by White and Kaliner[10] was used. Rabbit anti-human lactoferrin, 0.1 ml, diluted 1:1000 in 0.1 mol carbonate buffer, pH 9.6, was plated per well and allowed to adhere overnight at 4° C, or for 2 hours at 37° C. The wells were washed four times with phosphate-buffered saline solution and then were blocked for 30 minutes at 23° C with 250 μl of 1% goat serum diluted in PBSS. The next two additions were preceded by four washes with PBSS and followed by a 90-minute incubation at 37° C as follows: (1) 0.1 ml of tracheal aspirate or human lactoferrin standard, 1 to 100 ng/ml in PBSS, and (2) 0.1 ml anti-human lactoferrin conjugated to horse radish peroxidase and diluted 1:1000 in PBSS. Wells were washed four times with PBSS. The plates were developed and read as for the albumin assay. Measurements were made in triplicate and expressed as average micrograms per milliliter.

Lysozyme analysis. Lysozyme was measured by a turbidimetric assay on the basis of the enzymatic hydrolysis of bacterial cell walls.[11] Fifty microliters of lysozyme standard or sample was added to a 1 ml suspension of *M. lysodeikticus* (0.25 mg/ml in 0.1 mol phosphate-buffered saline solution, pH 7.0), and the decrease in absorbance per minute at 450 nm was recorded on a spectrophotometer (Hewlett-Packard Co., Palo Alto, Calif.). Measurements were made in duplicate and expressed as micrograms per milliliter.

Statistical analysis. Summary statistics were done with MEDLOG software and all other comparisons with BMDP (Biomedical Data Package) statistical software (University of California Press, Berkeley, 1989). The HMD and BPD group means for the demographic variables (birth weight and gestational age) were compared by *t* tests, and the dichotomous variables, gender and race, by chi-square analysis. Because of the relatively brief period of intubation for babies with HMD alone, matched daily tracheal aspirate samples were available for both BPD and HMD subjects only on days 1 to 4 of life. Comparisons are therefore limited to these early days of care. Additionally, as was true in this study, some babies with HMD do not undergo intubation until day 2, and the tube is removed from many infants with uncomplicated HMD by day 4 of life. Therefore not all babies could have samples obtained every day. This limitation was particularly true of babies in the HMD group on days 1 and 4. Unmeasured variables occurring on some of these days precluded standard repeated measures analysis; therefore the daily means of lactoferrin, lysozyme, and albumin for the BPD and HMD groups were compared by

Table II. Summary of patient demographic data

	Gender (M/F)	Birth weight (gm)	Gestational age (wk)	Oxygen (days)	Mechanical ventilation (days)
HMD (n = 18)	10/8	1956 ± 664* (900-3339)	33 ± 3* (28-36)	7 ± 3 (2-15)	5 ± 2 (1-10)
BPD (n = 18)	13/5	1318 ± 591 (690-2800)	30 ± 3 (26-36)	207 ± 242 (25-911)†	38 ± 45 (6-183)
Mild to moderate BPD (n = 10)	4/6‡	1361 ± 636 (690-2800)	30 ± 3 (27-36)	99 ± 157 (22-559)	16 ± 11 (6-45)
Severe BPD (n = 11)	10/1	1239 ± 490 (720-2030)	30 ± 3 (26-34)	325 ± 239 (45-911)†	60 ± 51 (14-183)

Values (except gender) are expressed as mean ± SD, with range in parentheses.
*p <0.05 by t test versus BPD group.
†One patient still receiving oxygen at end of study.
‡p <0.04 by Fisher Exact Test versus severe BPD group.

Mann-Whitney U tests with Bonferroni adjustment of the alpha statistic to correct for multiple comparisons. At each collection time the association of the concentration of a given individual mediator with the other measured mediators, fraction of inspired oxygen and peak inspiratory pressure, birth weight, and gestational age, were examined by using the Pearson product-moment correlation.

The patients with BPD were further subdivided into mild to moderate versus severe cases. Demographic variables were compared between the two groups by t test or Fisher Exact Test, as appropriate. For patients with BPD who had an endotracheal tube in place at least through day 13, overall trends of lactoferrin and lysozyme values with time were analyzed by averaging daily values during 4-day periods, testing for sphericity and orthogonal correlations, ranking data if appropriate, and then applying repeated measures analysis of variance. Correlation of mediator level with birth weight or gestational age was analyzed by linear regression, with mediator levels averaged for the first 14 days of life to obtain a uniform mediator level for each patient. Simple correlation of mediator levels with other simultaneously obtained mediator levels, ventilatory pressure limits, administered fraction of inspired oxygen, mean airway pressure, or BPD score was by Pearson correlation analysis. The relationship of the BPD score to the maximum or average mediator level for 28 days was evaluated with the Pearson correlation. For all analyses, statistical significance was considered with probability levels ≤0.05.

This study was approved by the investigational review board of Children's National Medical Center, Washington, D.C. Informed consent was not required, but an information sheet was provided for the parents.

RESULTS

Patient characteristics. Thirty-six infants with HMD were studied. Of these, 18 recovered from HMD and BPD developed in 18 (Table II). The BPD group had a signifi-

cantly lower mean birth weight and mean gestational age (p <0.05). The racial distribution of the BPD group (7 white, 11 black) was similar to that of the HMD group (10 white, 8 black). Of 36 mothers, one had received betamethasone 18 hours before the delivery of a baby in the HMD group.

An additional three babies in whom BPD developed were admitted to the nursery and entered into the study at 48 hours of age. They were not included in comparisons between HMD and BPD groups but were included in comparisons between mild-to-moderate and severe BPD groups. Thus, for analyses that distinguished severity of BPD, data on 21 patients were evaluated (Table II). Ten were classified as having mild to moderate BPD, with a median BPD score of 3 (range 2 to 4) and a median x-ray score of 4 (range 1 to 7). Severe BPD developed in 11 babies; the median BPD score was 6 (range 5 to 8) and the median x-ray score was 6 (range 4 to 8). The two groups had similar birth weights, mean gestational ages, and racial distributions (7 black and 4 white for the severe group vs 7 black and 3 white for the mild to moderate group). The severe group had significantly more boys than did the mild-to-moderate group (10% vs 3.6%; p <0.04).

Nine babies with severe BPD required steroids for the treatment of BPD (two began steroids during the study on days 11 and 13 of life, respectively), and four had repeated episodes of bronchospasm. One infant with severe BPD died of respiratory failure on day 45 of life. Three babies with mild to moderate BPD required steroids for the treatment of BPD (none during the time of sample collection), and one had repeated episodes of bronchospasm. Airway infection was not suspected or documented in any of the patients with HMD or BPD during the period of intubation and sample collection.

Enteral intake. Babies in the HMD group were not fed during the time of study. Of the 21 babies with BPD (two from the mild to moderate group and five from the severe
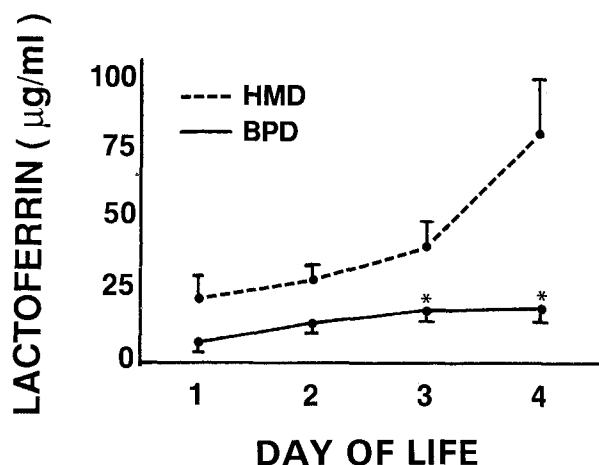
Fig. 1. Lactoferrin concentration in tracheal aspirates from babies in the HMD and BPD groups. Data represent mean ± SEM. HMD infants (*dashed line*): n = 6 on day 1; n = 14 on day 2; n = 11 on day 3; n = 6 on day 4. BPD infants (*solid line*): n = 10 on day 1; n = 13 on day 2; n = 15 on day 3; n = 13 on day 4. *$p < 0.05$ by Mann-Whitney U test with Bonferroni adjustment.

group), seven were given minimal volumes of formula or human milk during the last 3 to 7 days that they were studied; only one patient began feedings before 2 weeks of age.

Tracheal aspirate volume. No significant differences in the volume of tracheal aspirate recovered were noted between the HMD and BPD groups or among the infants in each group. There were no significant differences in recovered volume between babies who later had repeated bronchospasm and those who did not.

Lactoferrin. Mean lactoferrin concentrations in babies with BPD were less than in those with HMD on days 3 and 4 (Fig. 1). In babies with BPD, the mean lactoferrin concentration was low on day 1 (7 μg/ml) and remained consistently low (>19 μg/ml) during the first 4 days of life. In contrast, the mean lactoferrin concentration in babies with HMD was 23 μg/ml on day 1 and rose to 81 μg/ml on day 4. Lactoferrin concentrations in individual babies with BPD clustered at less than 30 μg/ml; concentrations in babies with HMD were higher, although more variable. On day 4, only one baby with HMD had a lactoferrin concentration <40 μg/ml; the levels in the remaining babies with HMD were >60 μg/ml.

For babies with BPD who continued to have an endotracheal tube in place, and for whom we had tracheal aspirate samples beyond day 4, lactoferrin concentrations remained consistently low; mean daily lactoferrin levels ranged from 7 to 35 μg/ml during days 1 to 28 of life. For the entire BPD group, lactoferrin levels tended to increase during the first 5 days (remaining <22 μg/ml), to remain relatively higher from days 8 to 18 (15 to 35 μg/ml), and then to decline to <20 μg/ml. For the first 16 days of life (Fig. 2), the increase

with time of the lactoferrin levels from the period of days 1 to 4 to the period of days 5 to 8 was greater for mild to moderate cases than for the severe cases ($p = 0.046$).

Lactoferrin levels did not correlate with the levels of albumin. The pattern of daily lactoferrin levels in individual babies did not correlate with clinical events such as extubation, pneumothorax, infection, or the administration of medications. Lactoferrin concentrations did not correlate with simultaneously recorded ventilator peak inspiratory pressure, mean airway pressure, or concentration of administered oxygen.

Lysozyme. Mean lysozyme concentrations (Fig. 3) were significantly less in babies with BPD (31 ± 5 μg/ml) than in babies with HMD (54 ± 7.5 μg/ml) only on day 3 ($p < 0.05$). Samples from day 4, which had the greatest difference between HMD and BPD groups, could not be submitted to statistical analysis because only four samples remained from the HMD group for lysozyme testing.

Combining HMD and BPD groups, and correcting for multiple tests, we found that lysozyme concentration significantly correlated with lactoferrin concentrations on days 1 ($r = 0.814$; $p < 0.01$) and 4 ($r = 0.813$; $p < 0.01$) of life.

For babies with BPD whose endotracheal tube remained in place beyond day 4 of life, lysozyme levels ranged from 13.7 to 109.1 μg/ml during the first 28 days of life. Levels tended to increase during the first week of life, and then to decrease. During the first 16 days of life, the lysozyme level was significantly lower ($p < 0.05$) in infants with severe BPD (n = 10) than in those with mild to moderate BPD (n = 6) for days 5 to 8 and days 9 to 12 (Fig. 4).

Lysozyme levels did not correlate with the levels of albumin or with clinical events such as extubation, pneumothorax, infection, or the administration of medications. There was also no correlation with simultaneously recorded ventilator peak inspiratory pressure, mean airway pressure, or concentration of administered oxygen.

Albumin. Mean albumin concentrations for days 1 through 4 fluctuated between 400 and 850 μg/ml and were similar for both HMD and BPD groups on each day. Albumin did not correlate with lactoferrin or lysozyme levels.

Mean albumin concentrations in all babies with BPD on days 1 to 28 of life ranged between 300 and 12,000 μg/ml with great patient-to-patient variability. When mild-to-moderate and severe groups were examined separately, albumin levels were not significantly different between the groups.

Polymorphonuclear cell count. Polymorphonuclear cells, which contain approximately 3 μg each of lactoferrin and lysozyme per 10^6 cells,[7] are a potential source of tracheal aspirate lactoferrin and lysozyme. Polymorphonuclear cell counts from days 1 to 4 of life were available for 23 babies (13 BPD, 10 HMD). In general, the numbers of cells recovered were not sufficient to account for levels of lacto-
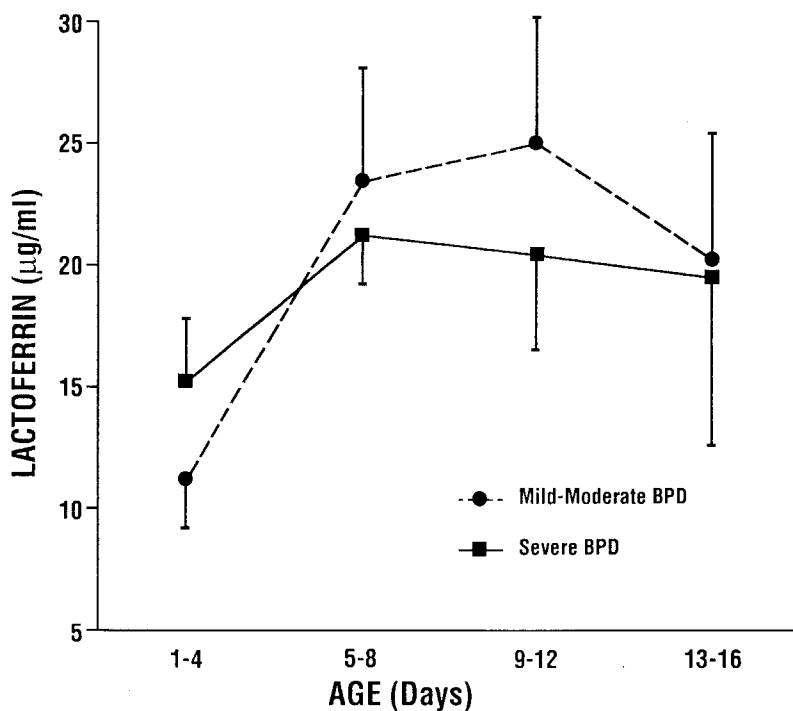
Fig. 2. Lactoferrin concentration in tracheal aspirates from infants with BPD. Data represent mean ± SEM. Mild to moderate BPD (n = 6): *dashed line*; severe BPD (n = 10): *solid line*.

ferrin and lysozyme seen (Table III). There was no correlation of the lactoferrin or lysozyme level with the number of polymorphonuclear cells recovered in the tracheal aspirate. Eight patients with BPD who were studied between day 4 and day 28 had polymorphonuclear cell counts ranging from 4.5×10^3 to 5×10^6 cells/ml.

Birth weight and gestational age. To examine whether differences in levels of lactoferrin and lysozyme reflected greater size or gestational age, we conducted regression analyses. Although higher lactoferrin levels were associated with higher birth weight and gestational age during the 28-day study period, this association did not hold true for the first 4 days of life. For lysozyme, higher levels correlated with higher birth weight and gestational age only on days 1 and 2, but not thereafter. Therefore the significant differences in lactoferrin and lysozyme levels between patients with HMD and those with BPD, and in lysozyme levels between mild-to-moderate and severe BPD groups, could not be related to birth weight or gestational age alone.

DISCUSSION

Lactoferrin is an iron-binding protein found in serous cells of airway submucosal glands[5] and contained in secondary granules of neutrophilic granulocytes.[7, 12] In the airway mucosa, the major source of lactoferrin is the serous cell of the submucous gland.[4, 13] Glandular secretions contain about 2% to 4% lactoferrin (as a percentage of total
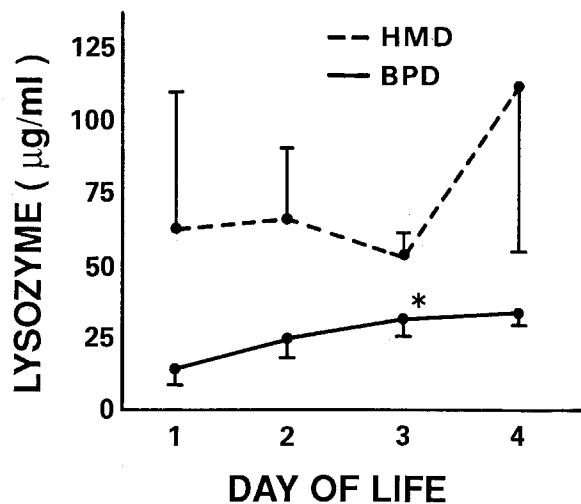

Fig. 3. Lysozyme concentration in tracheal aspirates from babies in the HMD and BPD groups. Data represent mean ± SEM. HMD infants *(dashed line)*: n = 5 on day 1; n = 14 on day 2; n = 10 on day 3; n = 4 on day 4. BPD infants *(solid line)*: n = 10 on day 1; n = 13 on day 2; n = 15 on day 3; n = 13 on day 4. *$^*p$ <0.05 by Mann-Whitney U test with Bonferroni adjustment.

protein),[4, 13] although these measurements have not heretofore been reported in tracheal aspirates from newborn infants. In recent studies employing human nasal secretions, lactoferrin was secreted by the glands in response to cholin-
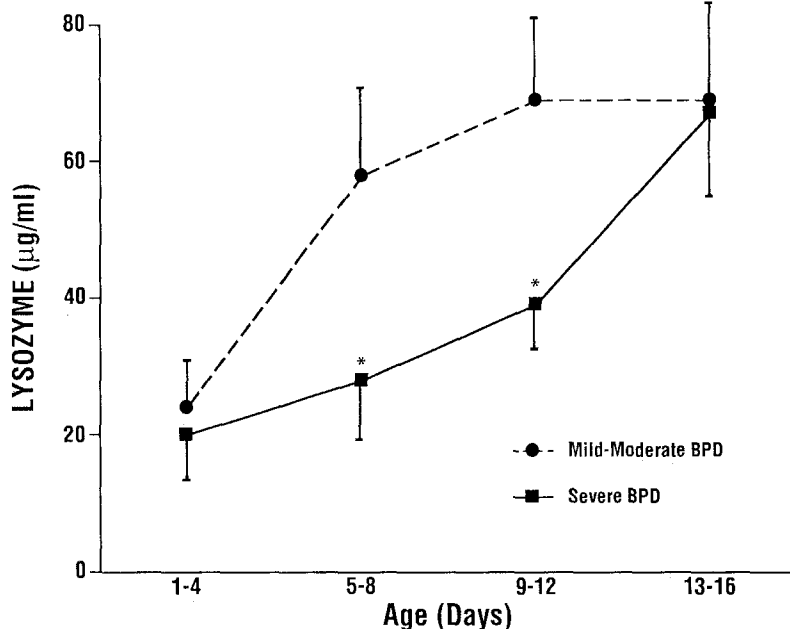
Fig. 4. Lysozyme concentration in tracheal aspirates from infants with BPD. Data represent mean ± SEM. Mild to moderate BPD (n = 6): *dashed line*; severe BPD (n = 10): *solid line. *p* <0.05 by repeated measures analysis of variance.

Table III. Tracheal aspirate polymorphonuclear cell count per milliliter of tracheal aspirate

		Polymorphonuclear cells	
	Day of life	Median ($\times 10^5$ cells)	Range
HMD (n = 10)	1	3.63	1.4×10^5 to 5.2×10^5
	2	1.93	1.9×10^5 to 2.0×10^5
	3	2.00	3.4×10^2 to 1.7×10^6
	4	4.50	7.5×10^2 to 2.8×10^6
BPD (n = 13)	1	1.39	4.3×10^2 to 5.9×10^6
	2	4.36	6.2×10^4 to 6.2×10^6
	3	6.50	5.0×10^2 to 4.3×10^6
	4	3.51	1.8×10^2 to 2.6×10^6

ergic stimulation,[14] in response to gustatory rhinitis,[15] as a reflex response after histamine stimulation,[16] and during the response evoked by topical allergen challenge.[17] Lactoferrin secretion in response to cholinergic stimulation is relatively reduced in nasal provocation of subjects with recurrent sinusitis,[14] suggesting the possibility that the inability to secrete serous cell products might predispose subjects toward recurrent respiratory infection. Lactoferrin, devoid of iron, protects rats in a dose-dependent manner against in vivo lung injury in a complement and neutrophil-dependent model system,[18] and has been proposed as an antioxidant protector of neonates.[19] Lactoferrin has also been proposed as a marker for inflammation, as a tool to assess granulocyte numbers or secretion,[7] and as a regulator of macrophage and granulocyte proliferation.[12]

Lysozyme is another antimicrobial protein found in nasal secretions and in many other external secretions. Lysozyme is the major protein in upper respiratory tract secretions, often representing 15% to 35% of total protein.[4, 13] Lysozyme is secreted along with lactoferrin by the serous cell. Lysozyme is a bactericidal protein that enzymatically degrades the bacterial cell walls of susceptible bacteria.

The significance of lactoferrin and lysozyme concentrations in tracheal aspirates of newborn infants with HMD is unclear, as is the relationship between inflammation and the development of BPD. It is possible that (1) decreased lactoferrin levels indicate an inability to mount an effective, and possibly beneficial, inflammatory response against acute lung injury; (2) lactoferrin may have a protective ef-

fect on the lung, preventing the development of BPD by its antibacterial or antioxidant properties; and (3) lactoferrin may be a marker for lung maturity with improved resistance to the development of BPD.

The source of lactoferrin and lysozyme in tracheal aspirates of newborn infants is not known for certain. One possible source might be neutrophils infiltrating the airways. The lactoferrin and lysozyme contents of neutrophils have each been measured at 3 μg/10^6 cells.[7] Merritt et al.[1] reported large numbers of inflammatory cells in tracheal samples from babies with HMD in whom chronic lung disease developed, in comparison with babies with HMD who recovered. If polymorphonuclear cells were a major source of airway lactoferrin or lysozyme, one might anticipate that levels of these proteins in babies with HMD would be lower than in babies with BPD. Our polymorphonuclear cell counts were not dissimilar to those of previous reports.[1, 2] However, the polymorphonuclear cell counts were similar for both babies with HMD and those with BPD, suggesting that the number of inflammatory cells cannot account for the relative deficiency of both lysozyme and lactoferrin in the babies in whom BPD developed. Moreover, the number of polymorphonuclear cells required to generate 25 to 100 μg of lysozyme and lactoferrin per milliliter of tracheal aspirate is well beyond the actual number of cells recovered. In addition, the ratio of lysozyme to lactoferrin was about 2:1 to 3:1 in the tracheal aspirates (the ratio that has been found in serous cell secretions[13]), and not 1:1, as found in lysate of polymorphonuclear cells. It is possible that some of the airway lactoferrin and lysozyme might be derived from neutrophils, but it seems certain that neutrophils cannot account for the high lactoferrin or lysozyme levels measured in tracheal aspirates.

Lactoferrin and lysozyme are present in serum in only small amounts.[20-23] In addition, we found no correlation between the levels of lactoferrin and the predominant plasma protein, albumin, in tracheal aspirates. Thus plasma is probably not an important source.

A third potential source of lactoferrin is formula or milk. In human milk, lactoferrin is found at approximately 1 mg/ml.[24] This source is not pertinent to our results because the babies with the highest levels of lactoferrin, the HMD group, were not fed during the duration of the study. None of the babies in the BPD group received a significant volume of feedings during the study period.

We believe that the most likely source of lactoferrin and lysozyme is airway submucous glands. Lactoferrin and lysozyme are secreted by serous cells of the submucous glands of the adult human bronchus[5] and nasal mucosa.[13] Glands of the lower respiratory tract of newborn infants are also the source of lactoferrin and lysozyme.[25] The relatively increased lactoferrin secretion by neonates who recover from HMD may reflect increased numbers or maturity of

serous cells or an increased capacity of the glands to secrete in response to inflammatory or physiologic stimuli. Because serous cells of tracheobronchial glands appear only near term gestation,[26] higher lactoferrin levels may also be a marker of relatively greater lung maturity. Neonates with relatively immature glandular development (as reflected in lower lactoferrin and lysozyme levels in tracheal aspirates) might thereby be prone to oxidant-induced lung injury and BPD.

Recent work, reported after the collections utilized for this study were completed, indicates that a major antioxidant in respiratory secretions is also a serous cell product.[27] The antioxidant, identified as uric acid,[27] is secreted along with lactoferrin and lysozyme in response to the same stimuli.[28] Thus uric acid and lactoferrin, both recognized antioxidants, are likely to be deficient in patients with BPD who have diminished serous cell function. The parallel deficiency of lactoferrin and lysozyme in the babies with BPD is strong evidence that serous cell malfunction is part of the disease. Whether the deficiency of uric acid and lactoferrin is part of the underlying milieu that predisposes infants to BPD is not yet certain, but our findings indicate the possibility that treatment with uric acid or lactoferrin might be useful in replacing deficient antioxidants.

Neonates with HMD and low lactoferrin concentrations in tracheal aspirates may be sufficiently at risk for the development of BPD to consider early therapeutic intervention when appropriate regimens become available. In addition, it may be possible in the future to regulate secretions from the submucosal glands pharmacologically to help facilitate the secretion of these antimicrobial factors.

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