The Influence of Gestation and Mechanical Ventilation on Serum Clara Cell Secretory Protein (CC10) Concentrations in Ventilated and Nonventilated Newborn Infants

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ABSTRACT: Clara cell secretory protein (CC10) is an important anti-inflammatory mediator in the adult lung, but its role in newborn pulmonary protection is uncertain. We examined the early postnatal behavior of CC10 in newborn serum and tracheal fluid and hypothesized that CC10 production is positively influenced by gestation. Blood from 165 infants from the first, third/fourth, and seventh days of life (gestational ages: 23–29 wk, 30–36 wk, >36 wk) and tracheal fluid (TF) from the first day of life from 32 ventilated infants were analyzed for CC10. Surfactant proteins A (SPA) and B (SPB) were also analyzed from the blood of a subgroup of infants. Serum CC10 on day 1 was highest in term infants (69.4 ng/mL), followed by moderately preterm (55.8 ng/mL), and then extremely preterm infants (median 42.1 ng/mL). Term infants also had higher tracheal fluid CC10 than preterm infants. (20.152 ng/mL *versus* 882 ng/mL). Mechanical ventilation increased serum CC10 only in moderately preterm infants, and only on d 1 [68.4 ng/mL *versus* 42.1 ng/mL (nonventilated moderately preterm infants)]. Serum CC10 decreased progressively by the end of the first week in all infants, in contrast to SPA and SPB, which increased. Our results show that CC10 is detectable in the blood of newborn infants and that a production surge occurs at birth. This surge is more pronounced in term infants and may confer them with superior extrauterine pulmonary protection compared with preterm infants. (*Pediatr Res* **60: 103–108, 2006**)

There has been little impact on the severity and incidence of chronic lung disease (CLD) despite considerable advances in perinatal care (1). The etiology of CLD, a syndrome of persistent respiratory insufficiency in which preterm infants continue to require supplemental oxygen and/or mechanical ventilatory support beyond the postconceptional age of 36 wk (2), remains uncertain, but there is increasing evidence to suggest that premature infants are unable to temper the inflammatory response caused by extrauterine insults such as mechanical ventilation, sepsis, and oxygen (3). An early increase of cellular and humoral inflammatory mediators (4,5),

as well as a concomitant decrease in protective antiprotease, antioxidant, surfactant, and anti-inflammatory systems (6), has been noted soon after birth in lung fluids of infants who subsequently developed CLD.

Recently, the 16-kD lung-specific anti-inflammatory protein, CC10 (known as CCSP, uteroglobin, or CC16), produced by nonciliated bronchiolar lung Clara cells, was shown to play a pivotal role in the prevention and amelioration of lung injury in adult mice and humans (7–9). Its effects on the newborn lung, however, are uncertain. The amniotic fluid and TF concentrations of CC10 increase with advancing gestational age (10–12), but whether this is a true representation of a gestational effect on CC10 production is questionable. For example, amniotic fluid studies, although involving large numbers of pregnancies, are predominantly composed of subjects with problematic and genetically abnormal pregnancies (10,11). Lung fluid studies, on the other hand, are usually small (12) and include only ventilated infants. The CC10 profile of normal infants is therefore not easily examined by either of these studies. Fortunately, CC10 is detectable in serum because it leaks passively from the lungs into the circulation through the bronchoalveolar blood barrier (13), and serum CC10 may therefore be used as a surrogate representative of lung CC10.

Serum CC10 has not been comprehensively studied in the neonatal population. There are also currently no data on gestational influence on serum CC10, and in this study, we sought to examine whether 1) CC10 was detectable in neonatal blood, 2) gestation and pulmonary outcome influences CC10 concentrations, and 3) there was any relationship between CC10 and the behavior of other pneumoproteins such as SPA and SPB.

We hypothesize that early production of CC10 during extrauterine adaptation is influenced by gestation. Additionally, our secondary hypothesis is that full-term infants will have

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Abbreviations CC10, Clara cell secretory protein, **CLD,** chronic lung disease, **RDS,** respiratory distress syndrome, **SP,** surfactant protein, **TF,** tracheal fluid, **TTN,** transient tachypnea of the newborn

higher concentrations of CC10 in both serum and TFs than premature infants at high risk of developing CLD.

METHODS

The infants in this study were recruited from the neonatal intensive care unit at the Royal Hospital for Women in Randwick, Australia, between June 2001 and December 2003. Infants were eligible for the study if they were less than 24 h of age at time of enrollment and if they required blood tests as part of routine medical management. Infants with major chromosomal anomalies, congenital anomalies of the lungs, or pulmonary hypoplasia were excluded. The infants were divided into three gestational cohorts consisting of extremely premature infants (23–29 wk gestation), moderately premature infants (30–36 wk gestation), and term infants (>36 wk gestation).

We obtained 0.5 mL of blood (by venous, arterial, or heel puncture) from each infant on d 1, 3–4, and 7 of life. Blood sampling was timed to coincide with medically indicated blood tests. D 7 specimens were not collected in term infants because most had been discharged from hospital by that time. Investigations were extended to include SPA and SPB assays during a later part of the study, in which an extra 0.2 mL of blood was obtained from consecutively enrolled infants for SPA and SPB measurement. TFs were collected from mechanically ventilated infants on the first day during routine pulmonary toilet: 0.5 mL of saline was instilled in the endotracheal tube and the residue suctioned after two to three ventilator breaths (6).

All specimens were immediately spun in an on-site laboratory for 10 min at 3500 RPM and the supernatant frozen and stored at –70°C until analysis. CC10 was assayed in serum and TF in batches of 40 in duplicates by a commercially available CC10 enzyme-linked immunosorbent assay (ELISA) (DIAMED, Belgium) (14). This uses anti-monoclonal antibody for quantitative determination of CC10. CC10 is then bound to specific biotin-conjugated antibodies, and the amount present is detected by addition of streptavidin–horseradish peroxidase, hydrogen peroxide, and tetramethylbenzidine buffer solution. Absorbency is measured at 450 nm and standard curves generated to determine CC10 concentrations. Detection limits are 0.03 ng/mL. The intra- and interassay coefficients of variation of the method are less than 4% at a level of 0.1 ng/mL and less than 3% at a level of 3 ng/mL (15). We confirmed in our laboratory that the CCSP standards used in the ELISA kit were of the same molecular weight as the recombinant human CC10 (rhCC10) (courtesy of April Pilon, Claragen, Baltimore, MD) and verified by Western blot that the ELISA kit antibodies recognized rhCC10.

CC10 assay values were expressed as weight per volume lavage fluid because an acceptable method for standardizing solute quantities in pulmonary lavage specimens has not been well established (16).

Serum SPA and SPB were measured in duplicate at four serial dilutions with ELISA inhibition assays by Flinders University, Adelaide, using previously established methods (17). Briefly SPA and SPB were first freed from any associated components using ethylenediaminetetraacetic acid, sodium dodecylsulfate, and Triton X-100. The proteins were then measured with ELISA inhibition assays using polyclonal antibodies raised against alveolar proteinosis–derived SPA and mature SPB, respectively. Absorbance was measured at 405 nm, and standard curves were generated to compute the concentration in each sample.

Demographic details recorded included antenatal steroids, maternal cigarette exposure, pregnancy and delivery histories, resuscitation, and ventilatory parameters.

Respiratory disease was diagnosed clinically and radiologically and included respiratory distress syndrome (RDS), transient tachypnea of the newborn (TTN), and other respiratory disorders. Infants with respiratory disease were treated according to nursery protocol, which included mechanical ventilation and oxygen for most extremely premature infants and continuous positive airway pressure for less premature infants with mild disease. Survanta®, which contains SPB but not SPA, was used in treatment of radiologically diagnosed RDS. Neonatal outcomes examined included mortality and the development of CLD, which was defined as the need for supplemental oxygen and/or ventilatory support at and beyond the postconceptional age of 36 wk in premature infants (2). No infant received steroids during the period of sample collection.

Variance of serum CC10 concentrations, obtained from a pilot sample of 28–32 wk gestation infants, was used for sample size calculations. This showed that 19 infants were required in each group to demonstrate adequate differences (to test for a mean difference of 5 ng/mL, alpha = 0.01, power = 0.90, two-tailed test). Categorical data were analyzed by Fisher's exact test and/or χ^2 test. Nonparametric analyses were used (Mann-Whitney *U*, Kruskal-Wallis, Friedman, Spearman's rho, and Wilcoxon). A $p < 0.05$ was considered significant. No infants were excluded from overall analyses but were excluded from CLD analysis if they died before 36 wk gestational age.

The Institutional Ethics Committee of the South East Sydney Area Health Service approved the study and signed informed consent was obtained from each infant's parents.

RESULTS

Blood samples ($N = 312$) were collected from 165 infants. Sixty were extremely premature (23–29 wk gestation; median 27 wk), 66 were moderately premature (30–36 wk gestation; median 33 wk), and 39 were full term (median 40 wk); demographic characteristics are summarized in Table 1. Only six term infants remained in the nursery by the end of the first week of life and therefore, d 7 blood results from this group were not included in our analysis. Investigations were extended in a later part of the study to include collection of an extra 0.2 mL of blood from a consecutive subpopulation of 41 infants (14 extremely preterm, 19 moderately preterm, and eight term) for serum SPA (97 samples) and SPB (95 samples) measurement. There was no difference in the demographics of the two groups.

Relationship between gestation, postnatal age, and serum CC10. The first collection of blood occurred at a median of 12 h (interquartile range: 3–19 h) after birth for all groups. D 1 CC10 concentrations were significantly influenced by gestation, being lowest in extremely preterm infants [median 42.1 ng/mL (17.2, 57.8)], followed by moderately preterm infants [55.8 ng/mL (26.0, 73.9)], and highest in term infants [69.4 ng/mL (59.6, 73.8); $p = 0.005$]. Subsequent serum CC10 concentrations stayed relatively stable in extremely preterm infants [d 4: 32.7 ng/mL (15.7, 51.6); d 7: 26.8 ng/mL (17.5, 44.6); $p = 0.738$] but fell significantly in the other two groups in comparison with d 1 [moderately preterm d 4: 31.2 ng/mL (19.0, 61.4); d 7: 28.9 ng/mL (14.7, 56.2), $p = 0.004$; term d 4: 25.2 ng/mL (15.4, 55.8); d 7: 23.9 ng/mL (13.7l, 34.0), $p = 0.006$] (Fig. 1).

Relationship between gestation and TF CC10. Of the 165 infants, 89 were ventilated and therefore eligible for TF collection. This was limited to office hours because of laboratory staff availability (samples had to be spun within an hour of collection). TF was collected from 49 infants on d 1, but 17 were rejected for testing because of an unsuitably small volume of return (less than 100 μL) (6). TFs were analyzed in 32 infants: 18 extremely premature, five moderately premature, and nine full term. There were no overall demographic biases between sampled infants and nonsampled ventilated infants.

TF CC10 on d 1 correlated positively with gestation (Spearman's rho correlation coefficient 0.410, $p < 0.020$). The median concentration in preterm infants was 882 ng/mL (442, 2655) [compare term infants: 20,152 ng/mL (724, 43, 112), $p = 0.022$].

Postnatal behavior of serum SPs. D 1 serum SPA concentrations from the subgroup of 41 infants (Fig. 2) were similar to those of serum CC10. Extremely preterm infants had lowest concentrations [0.7 μg/mL (0.6, 2.3)] followed by moderately preterm infants [1.0 μg/mL (0.6, 1.3)] and then term infants [1.1 μg/mL (0.67, 1.6), $p = 0.958$]. However, SPA concentrations rose on d 4 in all gestations, and this increase was most pronounced in more mature infants [0.8 μg/mL (0.6,

Table 1. *Demographic characteristics*

Gestational group	23–29 wk	30–36 wk	Full term	p
No. of infants	60	66	39	
Gestation (wk)	27 (25, 28)	33 (31, 34)	40 (38, 40)	<0.001
Antenatal variables				
Antenatal steroids	53 (88%)	31 (47%)	0 (0%)	<0.001
PIH/PET	8 (13%)	16 (24%)	2 (5%)	0.028
Chorioamnionitis	19 (32%)	8 (12%)	0 (0%)	<0.001
PROM	16 (27%)	15 (23%)	2 (5%)	0.025
Birth variables				
Birth weight (g)	1010 (780, 1252)	1942 (1658, 2333)	3357 (2966, 3708)	<0.001
Sex (male)	37 (62%)	33 (50%)	22 (56%)	0.406
Delivery (vaginal)	28 (47%)	16 (24%)	25 (64%)	0.001
Apgar score, 1 min	6 (4, 8)	8 (7, 9)	8 (4, 9)	0.001
Apgar score, 5 min	8 (6, 9)	9 (8, 9)	9 (7, 9)	0.010
Respiratory diseases				
RDS	48 (80%)	22 (33%)	1 (3%)	<0.001
TTN	8 (13%)	13 (20%)	11 (28%)	
Meconium aspiration syndrome			6 (15%)	
Pneumothorax			2 (5%)	
PPHN		1 (2%)	2 (5%)	
Others	2 (4%) (hydrops, apnea)		3 (8%) (TOF, apnea)	
Hypoxic ischemic encephalopathy			3 (8%)	
No respiratory illness, no support	2 (3%)	30 (45%)	11 (28%)	
Respiratory support				
Mechanical ventilation ± CPAP	49 (82%)	24 (36%)	16 (41%)	<0.001
CPAP only	7 (12%)	5 (8%)	0 (0%)	
Highest Fio$_2$ during first week	0.80 (0.35, 1.00)	0.28 (0.21, 0.45)	0.32 (0.21, 0.93)	<0.001
Surfactant treatment	42 (70%)	15 (23%)	3 (8%)	<0.001
HFOV therapy	10 (17%)	0 (1%)	3 (8%)	0.002
Nitric oxide therapy	9 (15%)	1 (2%)	3 (8%)	0.018
Patent ductus arteriosus	19 (32%)	6 (9%)	3 (8%)	0.001
Outcomes				
CLD	14 (23%)	1 (2%)	0 (0%)	<0.001
Death	15 (25%)	0 (0%)	0 (0%)	<0.001

Median (first, third quartiles) and number of infants (%) are shown.

PIH/PET, pregnancy-induced hypertension/preeclamptic toxemia; GBS, group B streptococcus; PROM, prolonged rupture of membranes; PPHN, persistent pulmonary hypertension of the newborn; TOF, tracheal esophageal fistula; CPAP, continuous positive airway pressure; FiO$_2$, fraction inspired oxygen; HFOV, high-frequency ventilation.
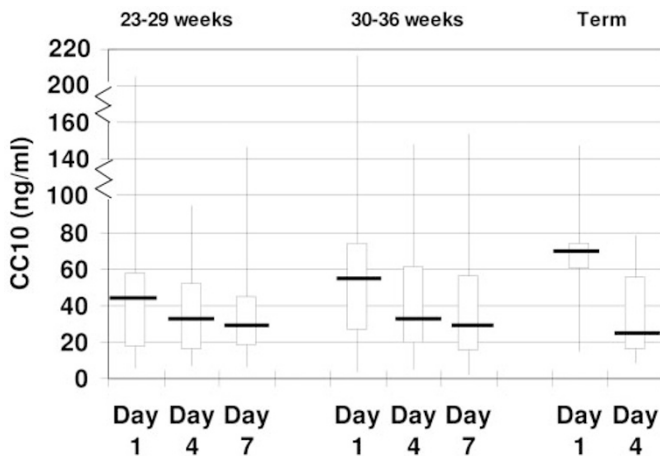

Figure 1. Relationship of CC10 to postnatal age: d 1, 4, and 7 serum concentrations. Median (*bars*), quartiles (*boxes*), and ranges (*vertical lines*) are shown. Extremely preterm number of infants ($n = 60$, $p = 0.738$); moderately preterm ($n = 66$, $p = 0.004$); term ($n = 39$, $p = 0.006$).
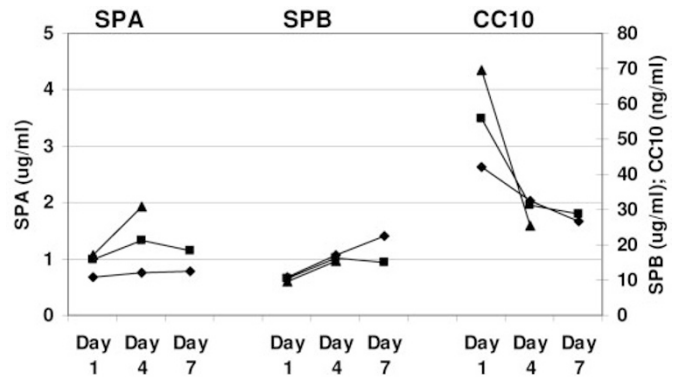

Figure 2. Postnatal pattern of serum pneumoproteins: Median concentrations of d 1, 4, and 7 are shown. ◆, term infants; ■, moderately preterm infants; ▲, extremely preterm infants.

Serum SPB varied more greatly than SPA, but no observable differences were seen in serum SPB between the gestational groups: extremely preterm: 10.7 μg/mL (6.9, 18.3), moderately preterm: 10.2 μg/mL (6.5, 16.8), and term: 9.5 μg/mL (3.1, 14.5) ($p = 0.757$). Serum SPB increased by almost twofold on d 4, but there was no apparent gestational influence (Fig. 2): 21.8 μg/mL (13.2, 33.5), 16.1 μg/mL (13.6, 22.9), and 15.3 μg/mL (10.7, 23.0), respectively ($p = 0.919$).

1.4), 1.3 μg/mL (0.9, 1.9), and 1.9 μg/mL (1.6, 2.4), respectively; $p = 0.008$].

Ten (24%) of the 41 infants had RDS, and all received surfactant treatment before collection of the d 1 samples.

Influence of ventilation and RDS on serum CC10 in corresponding gestation groups. Only four extremely premature infants did not require ventilatory support, 80% had RDS, and all but one were treated with Survanta®. Infants treated with Survanta® did not have statistically different concentrations of CC10 compared with those untreated [extremely preterms: d 1: 44.8 ng/mL (15.0, 63.2) *versus* 30.2 ng/mL (21.2, 55.2), $p = 0.742$; d 4: 24.2 ng/mL (15.6, 52.6) *versus* 37.6 ng/mL (28.6, 48.3), $p = 0.519$; moderately preterm: d 1: 55.4 ng/mL (43.3, 71.6) *versus* 55.2 ng/mL (24.7, 73.9), $p = 0.739$; d 4: 31.5 ng/mL (14.2, 38.3) *versus* 34.5 ng/mL (19.6, 65.4), $p = 0.297$).Ventilated infants had slightly higher d 1 serum CC10 than nonventilated infants [44.9 ng/mL (18.4, 60.5) *versus* 24.2 ng/mL (18.3, 27.2); $p = 0.257$]. Concentrations were also slightly higher in the 48 infants with RDS compared with infants without RDS on d 1 [49.3 ng/mL (16.0, 69.8) *versus* 12 non-RDS 24.0 ng/mL (20.8, 30.2), $p = 0.307$], but concentrations on d 4 and 7 were not different between the groups.

These differences were most marked in moderately preterm infants, in whom ventilation was associated with considerably higher d 1 CC10 concentrations [24 ventilated: 66.1 ng/mL (50.9, 78.5) *versus* 32 nonventilated: 41.8 ng/mL (23.9, 71.2); $p = 0.013$] (Fig. 3). Moderately preterm infants with RDS, however, had CC10 concentrations similar to those without RDS on d 1 of life [22 RDS infants: 55.4 ng/mL (43.1, 71.8) *versus* 44 non-RDS infants: 56.2 ng/mL (25.0, 74.7), $p = 0.173$].

There was no difference in CC10 concentrations between ventilated and nonventilated term infants [ventilated: 70.8 ng/mL (58.6, 82.4) *versus* nonventilated: 69.4 ng/mL (59.6, 73.8); $p = 0.682$]. The influence of RDS in this group was not analyzed because only one infant had RDS.

Maternal antenatal steroid treatment was examined as a confounder and was found to be an insignificant first day influence on preterm serum CC10 [extremely preterm maternal steroid: 42.1 ng/mL (15.7, 57.7) *versus* no maternal steroid: 55.7 ng/mL (24.0, 116.0), $n = 53$ (88%): $p = 0.266$; moderately preterm 56.9 ng/mL (39.5, 72.4) *versus* 55.5 ng/mL (24.7, 74.4), $n = 31$ (47%): $p = 0.876$].

Smoking history was obtained for mothers of 132 infants. Smoking did not influence serum CC10 on any days [E.G. d 1:

extremely preterm exposed: 55.2 ng/mL (22.0, 66.5) *versus* not exposed: 34.9 ng/mL (14.9, 56.2), $n = 11$ (22%), $p = 0.634$; moderately preterm exposed: 74.7 ng/mL (61.6, 77.3) *versus* not exposed: 57.3 ng/mL (36.5, 78.8), $n = 7$ (11%), $p = 0.278$; term exposed: 88.7 ng/mL (59.4, 118.1) *versus* not exposed: 69.3 ng/mL (57.9, 76.0), $n = 4$ (10%), $p = 0.766$].

Relationship between serum CC10 and CLD. Only one (2%) moderately preterm infant developed CLD. Fifteen (25%) of the 23–29 wk infants died, two from respiratory failure and one from pulmonary hemorrhage. Most deaths were from nonrespiratory causes, including intraventricular hemorrhages ($n = 7$), necrotizing enterocolitis ($n = 2$), meconium peritonitis ($n = 1$), hypotension ($n = 1$), and sepsis ($n = 1$). All the deaths occurred before 36 wk postconceptional age, and hence infants were excluded from analyses involving CLD. Fourteen (23%) infants developed CLD. All CLD infants and 19 of the 31 non-CLD infants were ventilated. Serum CC10 was slightly, but not significantly, lower on d 1 and 4 in CLD infants [d 1: 15.0 ng/mL (11.6, 72.4) *versus* 37.7 ng/mL (21.5, 56.2), $p = 0.500$; d 4: 21.6 ng/mL (15.2, 31.0) *versus* 33.8 ng/mL (22.5, 48.3), $p = 0.201$] (Fig. 4.).). A stronger trend was obtained if respiratory deaths were included in the analysis of CLD [d 1: 14.7 ng/mL (11.0, 72.2) *versus* 49.4 ng/mL (24.1, 57.8), $p = 0.116$; d 4: 20.4 ng/mL (14.1, 29.0) *versus* 37.4 ng/mL (22.7, 57.2), $p = 0.068$].

Relationship between serum pneumoproteins and renal function. Serum CC10, SPA, and SPB were analyzed in conjunction with serum creatinine to determine the effects of renal function. All proteins showed no significance on d 1, 4, and 7 when compared with serum creatinine.

DISCUSSION

To our knowledge, this is the first study to comprehensively demonstrate the behavior of serum CC10 in newborn infants during the first week of life. Apart from a small renal contribution, the majority of CC10 originates from the lungs (18), and one can assume that the CC10 that we analyzed from blood was a result of passive diffusion from the lungs to the circulation. Only minute amounts of CC10 cDNA have been found in human placentas and uteruses (19); therefore, it is unlikely that maternal contribution would be significant. Our
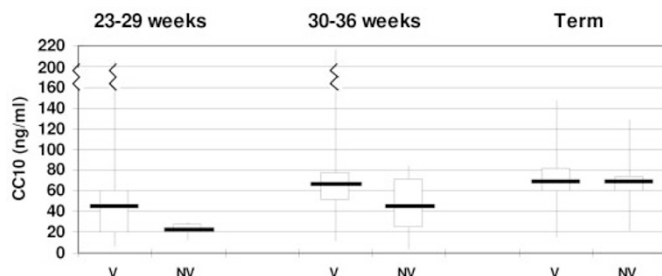
Figure 3. Influence of ventilation on serum pneumoproteins: d 1 concentrations of three gestational groups. Median (*bars*), quartiles (*boxes*), and ranges (*vertical lines*) are shown. V, ventilated; NV, nonventilated; 56 ventilated extremely preterm infants, four nonventilated extremely preterm infants, $p = 0.257$; 29 ventilated moderately preterm infants, 37 nonventilated moderately preterm infants, $p = 0.013$; 16 ventilated term infants, 23 nonventilated term infants, $p = 0.682$.
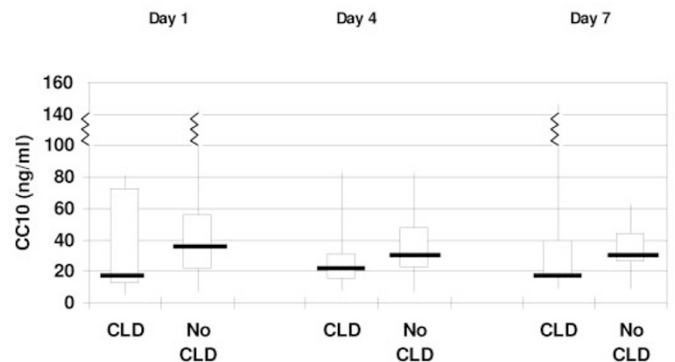
Figure 4. Relationship between serum pneumoproteins and CLD. D 1, 4, and 7 concentrations of extremely premature infants. Median (*bars*), quartiles (*boxes*), and ranges (*vertical lines*) are shown. Fourteen CLD infants; 46 infants with no CLD. D 1 $p = 0.500$, d 4 $p = 0.201$, d 7 $p = 0.259$.

results suggest that extremely preterm infants, especially those prone to CLD, produce less pulmonary CC10 than more mature infants with a good pulmonary outcome.

Differences in CC10 production may be vital in postnatal extrauterine pulmonary adaptation and in the development of the type of lung injury frequently associated with lung immaturity. CC10 is anti-inflammatory and inhibits phospholipase A_2 (PLA_2) activity (7), the production and function of interferon-γ (9), and the chemotaxis of fetal lung fibroblasts (7). The importance of CC10 in preventing lung injury has been demonstrated in genetically modified CC10-deficient mice, which have an increased sensitivity and exaggerated inflammatory response to lipopolysaccharide and hyperoxic or ozone-induced lung injury (20).

All the newborn infants in our study, regardless of lung disease, had much higher concentrations of serum CC10 than healthy adults (9–21 ng/mL) (21). Such concentrations may be necessary for acute postnatal lung adaptation. However, excessively elevated concentrations may also be an indication of a disrupted or underdeveloped bronchoalveolar blood barrier. For example, adults and animals with inflammatory lung disease and damaged pulmonary barriers have increased leakage of CC10 from the lungs into the circulation, as indicated by *decreased* pulmonary effluent and *increased* serum concentrations of CC10 (22). In our study, higher concentrations of serum CC10 in ventilated preterm infants may have been due to be a disruption of the bronchoalveolar blood barrier, either from lung disease or mechanical ventilation or from an underdeveloped barrier in extremely premature infants, but our TF studies confirmed that CC10 concentrations were, indeed, higher with increasing maturity.

We found that the postnatal behavior of serum CC10 was different from that demonstrated by the SPs; the latter increased toward the end of the first week instead of decreasing. The difference in behavior was not due to selection bias because a comparison of demographics showed no significant differences. This difference also cannot be solely explained by leakage because SPB is of a molecular size similar to that of CC10. The half-life of all three proteins in the circulation is less than 18 min (23), therefore discounting this as a factor.

The lack of gestational influence on serum SPA was an unexpected finding because fetal lung SPA has been shown to increase with advancing gestation (24). The molecular weight of SPA (35 kD) is considerably larger than that of CC10, and SPA may therefore be more confined to the intravascular space and not as dependent on glomerular filtration for clearance (23). However, we may have documented a difference in the behavior of serum SPA had we had obtained blood from more infants.

A gestational influence on CC10 production has been suggested by previous studies on amniotic fluid and TF of mechanically ventilated premature infants (10–12). CC10 is detectable in amniotic fluid from 15 wk of gestation (10) and increases with advancing gestation, possibly due to fetal airway growth (10,11). In a cross-sectional study of ventilated premature infants during the first 2 wk of life (median gestation 27.9 wk), CC10 concentrations in TF increased with both postnatal age and gestation (12). None of these studies, however, explored early postnatal changes. We studied infants from a wider gestational range and our longitudinal analysis included both nonventilated and healthy infants. Our results showed that term infants who are better equipped for extrauterine life had highest serum and TF CC10 on d 1; these new findings indicate that CC10 production at birth may be an important part of postnatal adaptation.

The influence of mechanical ventilation on serum CC10 concentration in premature infants is more difficult to delineate as this parameter is confounded by the two most important influences on serum CC10 concentrations: gestation and RDS. These two factors are inversely interdependent and in the extremely premature group, very few infants escaped mechanical ventilation and the majority had RDS. A small number had TTN and other disorders and were separated from the RDS group for analysis. In the moderately premature group, however, RDS did not make a difference to serum CC10 concentrations, even though a difference was seen between the ventilated and nonventilated infants. These results imply that CC10 production is independent of the surfactant system, as suggested by previous studies (11).

Elevated serum CC10 in ventilated preterm infants may have been caused by leakage through a damaged fragile preterm bronchoalveolar barrier. A proinflammatory response may have been incited by damage caused by mechanical ventilation, but this type of response usually takes a few days to weeks (25) and is not usually evident on the first day of life, as seen in the CC10 changes in this study, where serum CC10 continued to fall despite continued ventilation. We may not have found a demonstrably noticeable difference in serum CC10 between ventilated and unventilated term infants because of the heterogeneous variety of respiratory disease in this group. More studies, particularly in animal models, are required to investigate this.

This study also showed that CLD infants had the lowest serum CC10 concentrations compared with gestationally similar infants. Although infants at risk of developing CLD are usually ventilated, have RDS, and receive surfactant, these factors are not prerequisites for the development of CLD (26). When results for infants who died from respiratory causes were included in the analysis, serum concentrations of CC10 were even lower (although this difference was not significant: $p = 0.068$). The number of CLD infants in our cohort was small, and further studies with larger sample sizes of CLD-prone infants are required to explore the relationship between low levels of CC10 and CLD. This finding has important therapeutic implications as recombinant CC10 has been shown to confer significant pulmonary protection in newborn infants and animals (27,28).

We expressed CC10 concentrations as weight per volume lavage TF because an acceptable method for standardizing solute quantities in TF specimens is not well established (16). Adjustment for various markers can be performed; however, they have been found to be unreliable for the calculation TF dilution (29). Albumin is especially unsuitable as a denominator as there is little relation between serum and TF concentrations (29,30), the latter being also subject to variation because of acute flux and disease activity (30), particularly in

the context of heterogeneous lung diseases in our study population. The magnitude of CC10 difference in TF was much higher than anticipated from the serum levels. This may suggest enhanced CC10 production in full-term infants in response to lung disease.

In conclusion, our study has demonstrated that CC10 is detectable in blood of newborn infants and that production of CC10 is highest on the first day of life. We have also demonstrated that CC10 production is correlated to gestational age and that the importance of CC10 as an anti-inflammatory pneumoprotein places those with CC10 deficiency at possible risk of postnatal respiratory injury. CC10 production also appears independent of surfactant maturation, and further studies assessing the role of CC10 in extrauterine adaptation are urgently needed.

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