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Role of pulmonary infection in the development of chronic lung disease of prematurity

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ABSTRACT: We studied the role of ante- and post-natal infection in the development of chronic lung disease (CLD) of prematurity.

192 newborn infants (61 term and 131 pre-term of <34 weeks gestation: 88 with respiratory distress syndrome, 35 developed CLD and eight died) were recruited. 16S ribosomal RNA (rRNA) genes were identified by PCR of DNA isolated from 840 gastric and lung fluid samples. *Ureaplasma* spp. were also cultured.

Presence of 16S rRNA genes (OR 1.6, 95% CI 1.2–2.2) and *Ureaplasma* spp. (OR 3.6, 95% CI 1.7–7.7) was significantly associated with the development of CLD. This association remained if the 16S rRNA genes and *Ureaplasma* spp. were first identified within the first 3 days of life (OR 2.4 (95% CI 1.4–4.1) and 3.8 (95% CI 1.4–10.0), respectively) or if first identified after 3 days of age (OR 1.7 (95% CI 1.1–2.8) and OR 5.1 (95% CI 1.3–19.8), respectively). Peak lung fluid interleukin (IL)-6 and IL-8 were significantly associated with presence of microbes ($p<0.0001$ and $p=0.0001$, respectively) and development of CLD ($p=0.003$ and 0.001, respectively).

Both early and late microbial presence in neonatal lung fluid samples was significantly associated with the development of CLD suggesting that both ante- and post-natal infection play a role in the development of CLD.

KEYWORDS: Bronchopulmonary dysplasia, chronic lung disease of prematurity, infection, inflammation, 16S ribosomal RNA genes, *Ureaplasma* spp.

Chronic lung disease (CLD) of prematurity is a significant cause of morbidity and mortality in pre-term infants. Although its risk factors have been defined, its exact aetiology remains unknown. Recently, antenatal infection has received much attention [1, 2] in the development of CLD; however, it is uncertain if pulmonary post-natal infection, especially nosocomial infection, also has a role to play in the development of CLD. *Ureaplasma* spp., which are thought to be acquired antenatally, appear to be associated with the development of CLD [3, 4]. In their case–control study of 193 infants with birth weights <1,500 g, VAN MARTER *et al.* [5] reported an association between systemic infection acquired post-natally and development of CLD; findings later confirmed by HERNANDEZ-RONQUILLO *et al.* [6] in a smaller case–control study. Few studies have reported post-natal microbiological findings of pulmonary infection and their role in the development of CLD [7, 8]. Identification of microbes is hindered by current microbiological methods and by frequent use of antibiotics in this high-risk population, thus making identification of organisms difficult by standard culture methods. However, the identification of microbial presence by PCR amplification of prokaryotic 16S ribosomal RNA (rRNA) genes using universal bacterial primers shows promise, as reported by our group and others [8, 9]. We hypothesised that post-natal infection plays a role in the development of CLD. In this study: 1) we aimed to demonstrate whether there was an association between microbial presence identified by amplification of 16S rRNA genes; 2) for completeness, we also searched for *Ureaplasma* spp. in gastric and lung fluid samples by culture [9, 10]; 3) we also compared early (*i.e.* microbial presence within 3 days of birth) and late (*i.e.* microbial presence after 3 days from birth) to the development of CLD; 4) we also examined whether

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microbial presence in these samples was associated with increased pro-inflammatory cytokines namely interleukin (IL)-6 and IL-8, which we have previously shown to be increased in the presence of 16S rRNA genes [9].

METHODS

Patient groups

192 newborn infants were recruited from the Regional Neonatal Unit at University Hospital of Wales, Cardiff, UK, including 61 term (⩾37 weeks gestation) and 131 pre-term (⩽34 weeks gestation). In the pre-term group, 88 infants developed and recovered from respiratory distress syndrome (RDS): 35 developed CLD (defined pragmatically as oxygen dependence at 36 weeks post-menstrual age) and eight infants died before 28 days of age. Gentamicin and penicillin were used as first-line therapy in all infants who were suspected of respiratory illness on admission to the neonatal unit, and antibiotic therapy was modified according to the clinical status of the infant by the attending clinicians.

Sample collection

Gastric fluid was obtained wherever possible. Infants admitted to the neonatal unit routinely have a naso- or orogastric tube inserted for feeding and for deflating the stomach. Gastric fluid was obtained within 2 h of birth from infants who had not been fed previously, and was used for *Ureaplasma* spp. culture and isolation of DNA for identification of 16S rRNA genes and for the *Ureaplasma* spp. urease gene [7, 11]. Infants who received invasive mechanical ventilation for respiratory disease underwent nonbronchoscopic bronchoalveolar lavage (BAL), as described in the online supplement [12–15], or tracheal aspirates [16, 17] at the time of routine suction, as previously described. For those who received continuous positive airway pressure ventilation or were self-breathing in air or increased ambient oxygen, nasopharyngeal aspirates were obtained. Wherever possible, the lung fluid samples were obtained at the time of routine suction, especially in infants receiving invasive mechanical ventilation. Samples were obtained daily for the first week, then twice weekly until 28 days of age or until discharge, whichever occurred earlier. Samples were placed at 4°C until processing within 12 h of collection. The study was approved by the local research ethics committee (South East Wales Research Ethics Committee, Cardiff, UK) and written informed consent was obtained from the parents.

Detection of Ureaplasma spp. urease gene, and PCR and sequencing of 16S rRNA genes

DNA was extracted from samples using a commercially available DNA/RNA extraction kit (Qiagen, Crawley, UK). Sample integrity of extracted DNA was determined using PCR with primers designed against the human mitochondrial cytochrome oxidase gene, as previously described [18]. At least one satisfactory sample for DNA isolation to identify 16S rRNA genes was available from 164 infants. 16S rRNA gene-positive samples were identified using the primer sets 27f (AGA GTT TGA TC(AC) TGG CTC AG) and 1492r (TAC GG(CT) TAC CTT GTT ACG ACT T) to give the 1,465-bp product, and sequenced as previously described [19]. *Ureaplasma* spp. was determined by amplification of a 430-bp DNA product using primers U4 (ACG ACG TCC ATA AGC AAC T) and U5 (CAA TCT GCT CGT GAA GTA TTA C), as previously described [7, 20].

Culture-based detection of Ureaplasma spp.

25 μL of the sample was inoculated into 2 mL *Ureaplasma*-selective media (Mycoplasma Experience, Reigate, UK), incubated at 37°C and checked for an increase in pH for a period of 5 days [10]. Positive samples were confirmed with the *Ureaplasma*-specific PCR as described.

IL-6 and IL-8 ELISA

IL-6 and IL-8 were measured in duplicate in BAL fluid samples [9] from 63 infants, including 11 term infants, 21 from the RDS group, 24 who developed CLD and seven (six for IL-8) who died, by ELISA. Further details are given in the online supplement.

Statistical analyses

Nonparametric tests, including Mann–Whitney and Kruskal–Wallis, were used to compare two and multiple groups, respectively, and Fisher's exact test was used to compare tabular data (using SPSS version 15.0; IBM, Somers, NY, USA). A p-value of <0.05 was considered significant.

RESULTS

Patient characteristics

Patient characteristics are shown in table 1. Further details of admissions and causes of deaths are given on the online supplement. A total of 840 samples were obtained from 192 babies who were enroled in the study, including 61 who were born at term (gestation ⩾37 weeks) and 131 pre-term infants of ⩽34 weeks gestation, of which 88 developed and recovered from RDS, 35 developed CLD (defined pragmatically as oxygen dependence at 36 weeks post-menstrual age) and eight died. Of the 840 samples collected, 88 were from the term group, 408 from infants with RDS, 309 from the CLD group and 35 from babies who died.

Association between post-natal microbial genes and development of CLD

When the association of presence of pulmonary microbial genes and development of CLD samples was examined, 77% of the infants in the CLD group were positive compared with 48% of the RDS infants and 29% of term infants (OR 1.6, 95% CI 1.2–2.2; p=0.004; table 2). An association was noted between the presence of pulmonary *Ureaplasma* spp. and development of CLD, as shown in table 3. Of the 35 babies in the CLD group, 37% were positive for *Ureaplasma* spp., compared with 10% of RDS babies and 15% of term infants. When the pre-term groups who developed either RDS or CLD were compared, the association with development of CLD and presence of *Ureaplasma* spp. was highly statistically significant (OR 3.6, 95% CI 1.7–7.7; p=0.0004).

In order to determine if the microbes were acquired antenatally or nosocomially, we divided the samples into those that were first noted to be positive on or before 3 days of age or after 3 days of age. The association between day of detection of colonisation with *Ureaplasma* spp. and other microbes relative to development of CLD is shown in tables 4 and 5, respectively. There was a significant association between 16S rRNA genes and *Ureaplasma* spp. and development of CLD if

TABLE 1 Patient characteristics

	CLD	RDS	Term	Died
Patients	35	88	61	8
Samples	309	408	88	35
GF:BAL:NPA:ET	10:260:9:30	69:73:236:30	51:37:0:0	4:22:3:6
Gestational age weeks* **	26^{+5} (23^{+4}–31^{+6})	30^{+5} (26–34)	38^{+5} (37–42^{+1})	26^{+6} (25^{+6}–30^{+3})
Birth weight kg* **	1.03 (0.53–2.73)	1.50 (0.61–3.41)	2.92 (0.91–4.35)	0.87 (0.55–1.49)
Males:females	18:17	41:47	41:20	6:2
Vaginal:Caesarean delivery	19:16	24:64	33: 28	3:5
Antenatal steroids >24 h#	22 (63)	71 (81)	2 (3.3)	7 (88)
Surfactant#	35 (100)	43 (49)	1 (2)	8 (100)
pPROM	6 (17)	15 (17)	6 (10)	1 (13)
Ventilation IPPV:CPAP:SV	35:0:0	47:28:13	19:13:29	8:0:0

Data are presented as n, median (range) or n (%). CLD: chronic lung disease; RDS: respiratory distress syndrome; GF: gastric fluid; BAL: bronchoalveolar lavage; NPA: nasopharyngeal aspirate; ET: entotracheal aspirate; pPROM: prolonged pre-term rupture of membranes. IPPV: intermittent positive pressure ventilation; CPAP: continuous positive airway pressure; SV: self-ventilating. ***: $p<0.001$ when groups compared; #: $p<0.001$ when term infants compared to the CLD, RDS and died groups.

first identified within the first 3 days of life (OR 2.4 (95% CI 1.4–4.1; p=0.003) and OR 3.8 (95% CI 1.4–10.0; p=0.005), respectively) and also if first identified after 3 days of age (OR 1.7 (95% CI 1.1–2.8; p=0.036) and OR 5.1 (95% CI 1.3–19.8; p=0.01), respectively).

Relationship between IL-6 and IL-8 and microbial genes and clinical outcome

The peak results for each individual infant were correlated with the presence or absence of either 16S rRNA genes or *Ureaplasma* spp., as shown in figure 1a and b. Samples positive for either microbial genes or *Ureaplasma* spp. had median BAL fluid IL-6 and IL-8 values of 12,604 and 97,358 pg·mL^{-1}, respectively, which were six- and four-fold greater than the median values in the microbe-negative samples (2,074 and 21,970 pg·mL^{-1}, respectively; both $p<0.0001$). When only samples from the first 3 days of age were examined, a significant relationship was also noted between IL-6 and IL-8 concentrations (both $p<0.05$) and presence of microbes within the first 3 days of life (fig. 1c and d).

TABLE 2 Relationship between the presence of 16S ribsosomal RNA (rRNA) genes and clinical outcome

	16S rRNA genes detected	16S rRNA genes not detected	Total
Term	14 (29)	34 (71)	48
RDS	35 (48)	38 (52)	73
CLD	27 (77)	8 (23)	35
Died	2 (25)	6 (75)	8
Total	78	86	164

Data are presented as n (%) or n. p=0.004 for comparison for positive and negative 16S rRNA genes for the respiratory distress syndrome (RDS) and chronic lung disease (CLD) groups. Sufficient DNA could only be extracted from 48 out of 61 term infants and 73 out of 88 RDS infants, due to limited cell numbers.

The peak values for the IL-6 and IL-8 for each diagnostic group, together with presence of absence of microbial presence or absence, are shown in figure 2. BAL fluid samples from the term group had the lowest IL-6 and IL-8 median values of 980 and 12,107 pg·mL^{-1}, respectively. RDS infants had median IL-6 and IL-8 levels of 5,170 and 17,502 pg·mL^{-1}, respectively, whereas CLD babies had median levels of 15,594 and 122,550 pg·mL^{-1} ($p<0.005$ and 0.0001, respectively, comparing RDS *versus* CLD groups).

Microbes associated with peaks in IL-6 and IL-8

Amplicons from 16S rRNA gene-positive samples that coincided with peaks in IL-6 and IL-8 were sequenced to determine the predominant microbial species (table 6). *Staphylococcus epidermidis* was the most prevalent organism that was isolated from infants who went on to develop RDS, with one peak of IL-6/IL-8 being associated with *Fusobacterium nucleatum*. Gram-negative organisms were more frequently associated

TABLE 3 Relationship between the presence of *Ureaplasma* spp. and clinical outcome

	Ureaplasma spp. detected	*Ureaplasma* spp. not detected	Total
Term	9 (15)	52 (85)	61
RDS	9 (10)	79 (90)	88
CLD	13 (37)	22 (63)	35
Died	3 (38)	5 (62)	8
Total	34	158	192

Data are presented as n (%) or n. p=0.0004 for *Ureaplasma* positive and negative for the respiratory distress syndrome (RDS) and chronic lung disease (CLD).

TABLE 4 Comparison of the presence of 16S ribosomal RNA (rRNA) genes with first positive identification at ⩽3 days of age or >3 days of age with negative samples

	16S rRNA gene-positive ⩽3 days	16S rRNA gene-positive >3 days	16S rRNA gene-negative	Total
RDS	14 (19)	21 (29)	38 (52)	73
CLD	14 (40)	13 (37)	8 (23)	35
Total	28	34	46	108

Data are presented as n (%) or n. Comparisons were made between the early and late groups with the 16S rRNA gene-negative group. RDS: respiratory distress syndrome; CLD: chronic lung disease.

with IL-6/IL-8 peaks in CLD infants and included *Escherichia coli*, *Haemophilus influenzae*, *Enterobacter* spp. and *Pseudomonas aeruginosa*, although Gram-positive bacteria, including *S. epidermidis* and *Staphylococcus aureus* were also identified. Twice as many *Ureaplasma parvum* (two RDS and four CLD) were isolated relative to *Ureaplasma urealyticum* (one RDS and two CLD) in both RDS and CLD babies.

DISCUSSION

In this study, we examined >800 lung and gastric fluid samples from 192 newborn infants, and identified microbial genes using universal bacterial PCR primers to assess the role of post-natal infection in the development of CLD. We also investigated whether the pro-inflammatory cytokines IL-6 and IL-8 were increased, in order to suggest whether the microbial gene presence was indeed likely to be an infective process rather than a colonisation one. The results show that the presence of 16S rRNA genes was increased in babies developing CLD when compared with those who did not; the association was greatest when the microbial genes were detected within the first 3 days of life, but remained significant even when 16S rRNA genes were first detected beyond 3 days of age. The microbial presence is likely to be due an infective process, as both IL-6 and IL-8 were markedly increased in BAL fluid samples with microbial genes detected. Finally, we confirm previous findings implicating *Ureaplasma* in the development of CLD [3, 4].

Post-natal infection is strongly suspected in the development of CLD. VAN MARTER *et al.* [5], in a case–control study, identified post-natal systemic infection, as assessed by systemic features, as a risk factor for the development of CLD. Similar findings have been reported by HERNANDEZ-RONQUILLO *et al.* [6] and, more recently, LAHRA *et al.* [21]. However, these studies used epidemiological methods to implicate post-natal systemic infection in the development of CLD. Few studies have prospectively investigated this association of post-natal pulmonary infection and CLD by identification of microbial genes as we have in this study with a large number of prospectively collected samples from 192 newborn infants. Our findings suggest that 16S rRNA genes if first identified within 3 days of birth, suggesting antenatal acquisition, were strongly associated with the development of CLD. The association remained if microbes were first detected beyond 3 days of age, suggesting post-natal acquisition.

The presence of pulmonary *Ureaplasma* spp. was similarly associated with the development of CLD; again, the association remaining strongest for samples collected within 3 days of birth. *Ureaplasma* spp. were detected in 13 (37%) out of 35 babies who developed CLD, compared with nine (10%) out of 88 of RDS babies and nine (15%) out of 61 term infants. The association between the presence of pulmonary *Ureaplasma* spp. and development of CLD remained if it was first acquired after 3 days of age (table 4). Current evidence, however, suggests that *Ureaplasma*, even if first identified at few days of age, is likely to be acquired antenatally. These data support the conclusions of the meta-analyses by both WANG *et al.* [3] and SCHELONKA *et al.* [4] that there is an association between the development of CLD and presence of pulmonary colonisation of this organism. Nevertheless, controversy persists regarding the relevance of pulmonary colonisation by *Ureaplasma* in preterm infants, as increased vaginal colonisation is observed in mothers who deliver pre-term when compared with those who deliver at term [22]. Its true significance in the development of CLD can only be confirmed by an adequately powered randomised control trial to determine if its eradication can

TABLE 5 Comparison of the presence of *Ureaplasma* spp. with first positive identification at ⩽3 days of age or >3 days of age with negative samples

	Ureaplasma spp. positive age ⩽3 days	*Ureaplasma* spp. positive age >3 days	*Ureaplasma* spp. negative	Total
RDS	6 (7)	3 (3)	79 (90)	88
CLD	8 (23)	5 (14)	22 (63)	35
Total	14	8	101	123

Data are presented as n (%) or n. RDS: respiratory distress syndrome; CLD: chronic lung disease.

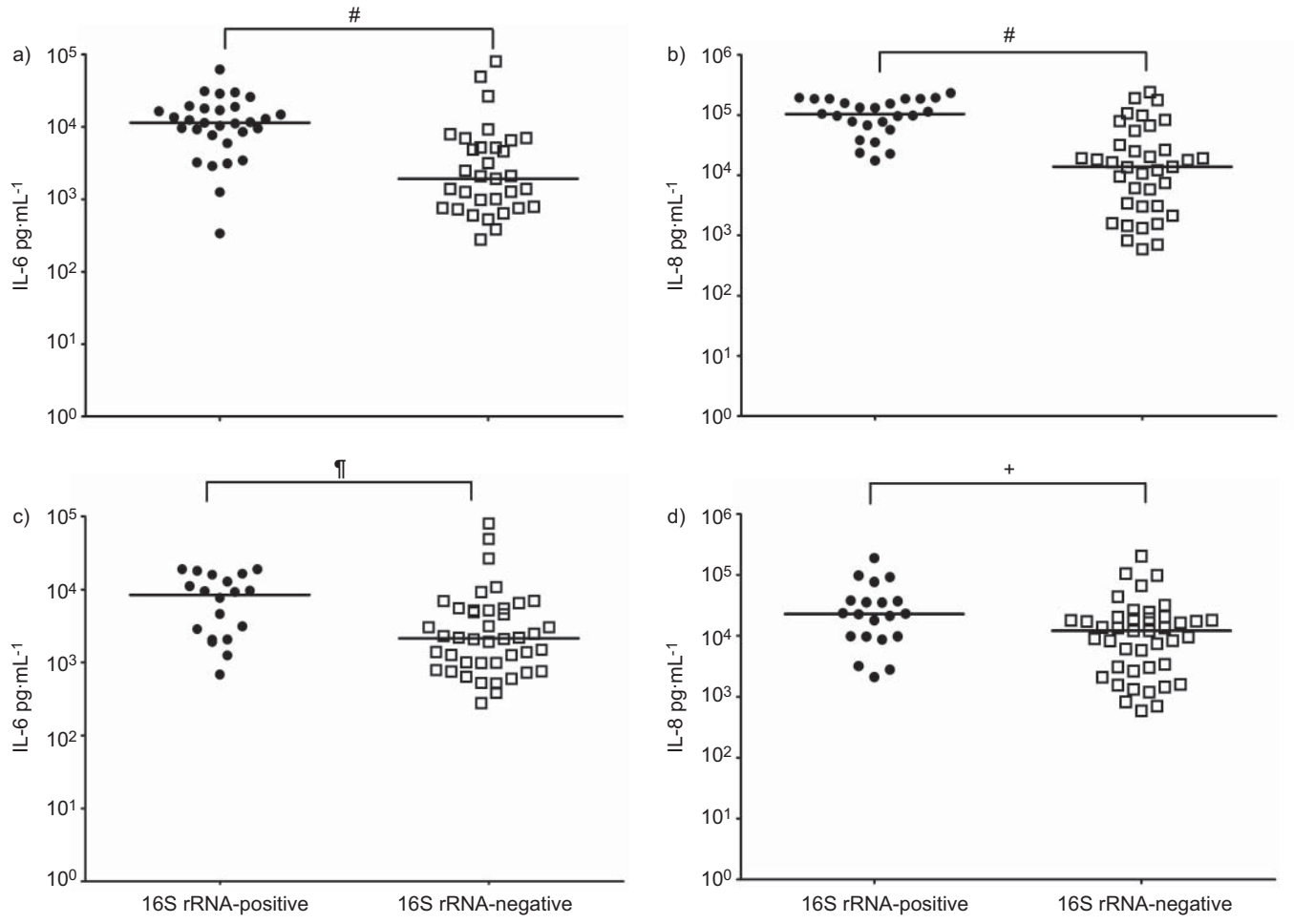

FIGURE 1. Association between peak a) interleukin (IL)-6 and b) IL-8 concentrations, and presence of microbial genes in bronchoalveolar lavage fluid samples. Peak c) IL-6 and d) IL-8 within 3 days of age. IL-6 and IL-8 were $\log_{10}$ transformed. rRNA: ribosomal RNA. #: $p<0.0001$; ¶: $p=0.015$; +: $p=0.016$.

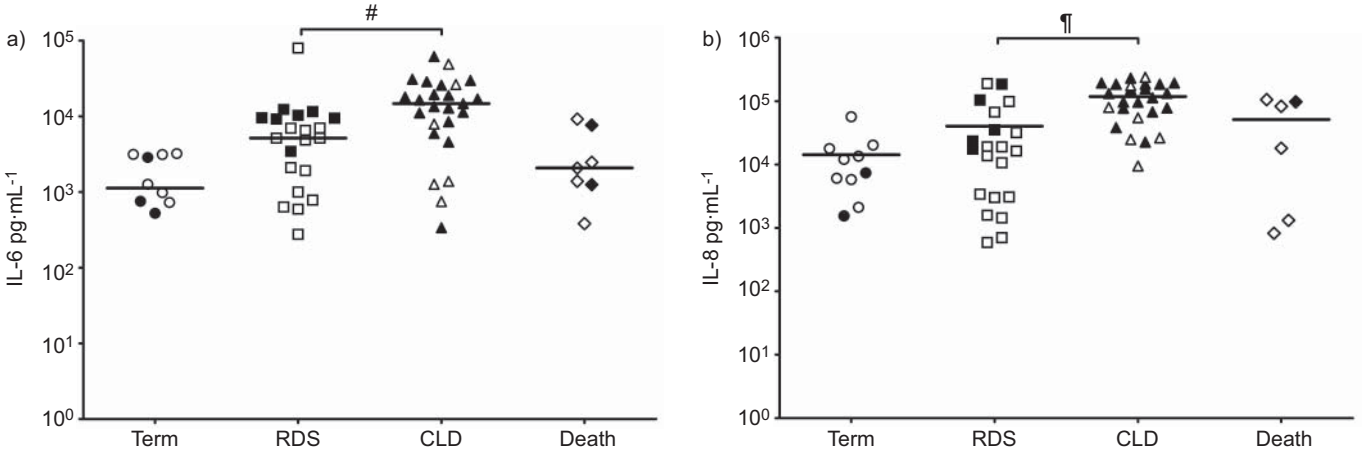

FIGURE 2. Peak levels of a) interleukin (IL)-6 and b) IL-8 from bronchoalveolar lavage samples for term, respiratory distress syndrome (RDS) and chronic lung disease (CLD) infants, and those who died. Open symbols show infants negative for 16S ribosomal RNA (rRNA) genes and closed shapes represent infants positive for 16S rRNA genes. IL-6 and IL-8 were $\log_{10}$ transformed. #: $p<0.005$; ¶: $p<0.0001$.

TABLE 6 Microbes associated with peak concentrations of interleukin (IL)-6 and IL-8 from bronchoalevolar lavage (BAL) fluid samples

	Samples
CLD	
Gram-positive	
Staphylococcus epidermidis	4
Streptococcus haemolyticus	1
Gram-negative	
Escherichia coli	2
Haemophilus influenzae	1
Enterobacter spp.	1
Pseudomonas aeruginosa	1
Other	
Ureaplasma parvum	4
Ureaplasma urealyticum	2
Mixed	2
RDS	
Gram-positive	
Staphylococcus epidermidis	3
Gram-negative	
Fusobacterium nucleatum	1
Other	
Ureaplasma parvum	2
Ureaplasma urealyticum	1

Data are presented as n. Peak levels of IL-6 and IL-8 from BAL fluids from pre-term infants were correlated with the presence of infection within a day of the peak value. 16S ribosomal RNA gene-positive samples were then sequenced to identify the infecting organism. CLD: chronic lung disease; RDS: respiratory distress syndrome.

decrease the rates of CLD [23, 24]. Our data also suggest that late manifestation of *Ureaplasma* spp., whether acquired antenatally or not, will need to be considered in the causation of CLD and be accounted for in any future interventional trials investigating if eradication of pulmonary *Ureaplasma* spp. decreases the rates of CLD.

An interesting observation was that 41% of infants born at 25 weeks were colonised with *Ureaplasma* spp., all of whom developed CLD, whereas 42% of those born at 26 weeks were colonised but only 18% developed CLD. This implies that the most immature infants are at risk of acquiring early microbes; whether this is due to immaturity of the infant's immune system is speculative. Furthermore, the vast majority of the microbes were acquired in the first 3 days of age, thus also suggesting that the vast majority of infections are likely to be acquired *in utero*, although in this study, no differences were noted for pre-term prolonged rupture of membranes. Unfortunately, comprehensive data on placental histology was not available to further investigate this early association between the development of CLD and chorioamnionitis, although our previous study did support this association [9].

The presence of microbial genes may simply reflect pulmonary colonisation, especially in those infants who have an endotracheal tube *in situ*. Thus, although it has limitations, we measured IL-6 and IL-8 in BAL fluid from those infants who underwent nonbronchoscopic BAL [13–15] to minimise contamination. The increased BAL fluid IL-6 and IL-8 implies that pulmonary inflammation is increased in babies who were positive for microbial genes, thus suggesting an infective process rather than microbial colonisation. We have previously reported this association between IL-6 and IL-8 with 16S rRNA gene positivity and development of CLD by examining intrauterine tissue samples and BAL fluid from a cohort of 41 pre-term infants [9]. Furthermore, we have previously shown that concentrations of IL-6 and IL-8 were significantly higher on the first day of life in *Ureaplasma*-infected babies compared with those who were negative [7]. The significance of high IL-8 levels in BAL samples could result in the recruitment of neutrophils, the predominant inflammatory cell in CLD lungs [13]. The viability of the isolated *Ureaplasma* spp. was confirmed by culture but the presence of 16S rRNA genes may simply reflect nonviable organisms, as these infants, on the whole, were on regular antibiotic treatment during the first week of life, especially the pre-term ones.

It was interesting to note that the CLD infants were colonised more frequently with more virulent organisms, including Gram-negative ones (*e.g. E. coli, H. influenzae*, *Entrobacter* spp. and *P. aeruginosa*), than infants with RDS. The pro-inflammatory nature of the Gram-negative endotoxin lipopolysaccharide (LPS) has been shown, in a pre-term lamb model, to contribute to lung injury [25]. Ventilated lambs born to ewes who had received intra-amniotic LPS had increased inflammatory markers and fewer alveoli compared with ventilated controls. *S. epidermidis* was prevalent in a number of both RDS and CLD infants. Although it is often thought to be a commensal and contaminant, LAHRA *et al.* [21] reported that post-natal systemic infection with *S. epidermidis* had similar effect on the development of CLD as more virulent organisms.

This study has added further evidence for the role of post-natal infection in development of CLD. It highlights the possible significance of antenatal acquisition of infection by determining that the presence of organisms within the first 3 days of life correlated with high levels of inflammatory mediators IL-6 and IL-8. It also suggests that nosocomially acquired infection is also likely to contribute to the development of CLD.

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STATEMENT OF INTEREST

A statement of interest for P.L. Davies can be found at www.erj.ersjournals.com/site/misc/statements.xhtml

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REFERENCES

1 Maxwell N, Davies PL, Kotecha S. Review of the role of antenatal infection in the pathogenesis of chronic lung disease of prematurity. *Curr Opin Infect Dis* 2006; 19: 253–258.

2 Been JV, Zimmermann LJ. Histological chorioamnionitis and respiratory outcome in preterm infants. *Arch Dis Child Fetal Neonatal Ed* 2009; 94: F218–F225.
3 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.
4 Schelonka RL, Katz B, Waites KB, *et al.* Critical appraisal of the role of *Ureaplasma* in the development of bronchopulmonary dysplasia with metaanalytic techniques. *Pediatr Infect Dis J* 2005; 24: 1033–1039.
5 Van Marter LJ, Dammann O, Allred EN, *et al.* Chorioamnionitis, mechanical ventilation, and postnatal sepsis as modulators of chronic lung disease in preterm infants. *J Pediatr* 2002; 140: 171–176.
6 Hernandez-Ronquillo L, Tellez-Zenteno JF, Weder-Cisneros N, *et al.* Risk factors for the development of bronchopulmonary dysplasia: a case-control study. *Arch Med Res* 2004; 35: 549–553.
7 Kotecha S, Hodge R, Schaber JA, *et al.* Pulmonary *Ureaplasma urealyticum* is associated with the development of acute lung inflammation and chronic lung disease in preterm infants. *Pediatr Res* 2004; 55: 61–68.
8 Oue S, Hiroi M, Ogawa S, *et al.* Association of gastric fluid microbes at birth with severe bronchopulmonary dysplasia. *Arch Dis Child Fetal Neonatal Ed* 2009; 94: F17–F22.
9 Miralles R, Hodge R, McParland PC, *et al.* Relationship between antenatal inflammation and antenatal infection identified by detection of microbial genes by polymerase chain reaction. *Pediatr Res* 2005; 57: 570–577.
10 Beeton ML, Chalker VJ, Maxwell NC, *et al.* Concurrent titration and determination of antibiotic resistance in *Ureaplasma* species with identification of novel point mutations in genes associated with resistance. *Antimicrob Agents Chemother* 2009; 53: 2020–2027.
11 Blanchard A, Hentschel J, Duffy L, *et al.* Detection of *Ureaplasma urealyticum* by polymerase chain reaction in the urogenital tract of adults, in amniotic fluid, and in the respiratory tract of newborns. *Clin Infect Dis* 1993; 17: Suppl. 1, S148–S153.
12 Kotecha S, Wangoo A, Silverman M, *et al.* Increase in transforming growth factor-β1 concentration in bronchoalveolar lavage fluid obtained from infants with chronic lung disease of prematurity. *J Pediatr* 1996; 128: 464–469.
13 Kotecha S, Chan B, Azam N, *et al.* Increase in interleukin-8 and soluble intercellular adhesion molecule-1 in bronchoalveolar lavage of premature infants with chronic lung disease. *Arch Dis Child* 1995; 72: F90–F96.
14 Kotecha S, Wilson L, Wangoo A, *et al.* Increase in interleukin-1 beta and interleukin-6 in bronchoalveolar lavage fluid obtained from infants with chronic lung disease of prematurity. *Pediatr Res* 1996; 40: 250–256.
15 de Blic J, Midulla F, Barbato A, *et al.* Bronchoalveolar lavage in children. ERS Task Force on bronchoalveolar lavage in children. *Eur Respir J* 2000; 15: 217–231.
16 Aghai ZH, Kode A, Saslow JG, *et al.* Azithromycin suppresses activation of nuclear factor-kappa B and synthesis of pro-inflammatory cytokines in tracheal aspirate cells from premature infants. *Pediatr Res* 2007; 62: 483–488.
17 Choi CW, Kim BI, Kim HS, *et al.* Increase of interleukin-6 in tracheal aspirate at birth: a predictor of subsequent bronchopulmonary dysplasia in preterm infants. *Acta Paediatr* 2006; 95: 38–43.
18 Fry NK, Tzivra O, Li YT, *et al.* Laboratory diagnosis of pertussis infections: the role of PCR and serology. *J Med Microbiol* 2004; 53: 519–525.
19 Davies PL, Spiller OB, Beeton ML, *et al.* Relationship of proteinases and proteinase inhibitors with microbial presence in chronic lung disease of prematurity. *Thorax* 2010; 65: 246–251.
20 Blanchard A. *Ureaplasma urealyticum* urease genes; use of a UGA tryptophan codon. *Mol Microbiol* 1990; 4: 669–676.
21 Lahra MM, Beeby PJ, Jeffery HE. Intrauterine inflammation, neonatal sepsis, and chronic lung disease: a 13-year hospital cohort study. *Pediatrics* 2009; 123: 1314–1319.
22 Kafetzis DA, Skevaki CL, Skouteri V, *et al.* Maternal genital colonization with *Ureaplasma urealyticum* promotes preterm delivery: association of the respiratory colonization of premature infants with chronic lung disease and increased mortality. *Clin Infect Dis* 2004; 39: 1113–1122.
23 Mabanta CG, Pryhuber GS, Weinberg GA, *et al.* Erythromycin for the prevention of chronic lung disease in intubated preterm infants at risk for, or colonized or infected with *Ureaplasma urealyticum*. *Cochrane Database Syst Rev* 2003; 4: CD003744.
24 Maxwell N, Nuttall D, Kotecha S. Does *Ureaplasma* spp. cause chronic lung disease of prematurity: ask the audience? *Ear Hum Develop* 2009; 85: 291–296.
25 Kramer BW, Kramer S, Ikegami M, *et al.* Injury, inflammation, and remodeling in fetal sheep lung after intra-amniotic endotoxin. *Am J Physiol Lung Cell Mol Physiol* 2002; 283: L452–L459.