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Role of *Ureaplasma* species in Neonatal Chronic Lung Disease: Epidemiologic and Experimental Evidence

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Abstract

The contribution of *Ureaplasma* respiratory tract colonization to the pathogenesis of bronchopulmonary dysplasia in preterm infants has been debated for over 20 years. We review the current understanding of the role of inflammation in altered developmental signaling in the preterm lung and the evidence from human studies and experimental models that *Ureaplasma*-mediated inflammation produces the BPD phenotype. We propose that *Ureaplasma* infection initiated *in utero* and augmented postnatally by exposure to volutrauma and oxygen elicits a sustained, dysregulated inflammatory response in the immature lung that impairs alveolarization, and stimulates myofibroblast proliferation and excessive collagen and elastin deposition. Potential strategies to prevent or ameliorate the effects of *Ureaplasma* infection *in utero* and in the preterm lung are discussed.

With recent improvements in perinatal care, bronchopulmonary dysplasia (BPD) has become a disease limited to the most immature infants (1), occurring in 30% of infants ≤28 wks gestation (2). Compared to the lung histology observed in the ventilated preterm lung during the pre-exogenous surfactant era, the ‘new’ BPD is characterized by more uniform inflation, fewer but larger alveoli, and less fulminant, but persistent inflammation (3). Studies of human infants and experimental animal models indicate that the central event in BPD pathogenesis is the interruption of normal developmental signaling during early stages of lung development by lung injury that is often initiated *in utero* by intrauterine infection and a subsequent dysregulated inflammatory response (1).

In 1988, three independent groups published single center cohort studies linking respiratory tract colonization with the mycoplasma species *Ureaplasma urealyticum* with the development of BPD in preterm infants (4–6). The studies differed in eligibility criteria, culture sites (eye, throat, vagina, rectum (4), stomach (5), nasopharynx (5,6), trachea (5,6), and blood (6)), and sampling frequency, but each study contributed important observations concerning *Ureaplasma* colonization in the preterm population. They observed that colonization 1) was inversely related to gestational age (4–6), 2) occurred in some infants delivered with intact membranes, suggesting *in utero* acquisition (6), and persisted in some infants until discharge (4). Twenty years later, more than30 additional studies with inconsistent results, 2 meta-analyses (7,8), and 3 comprehensive reviews (9–11) have been published without resolving the controversy of the importance of *Ureaplasma* respiratory tract colonization to the development of BPD, and no effective prevention or treatment strategies have been developed. This review will summarize the epidemiologic and

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experimental evidence that support a causative role of *Ureaplasma* spp. in BPD and explore potential therapeutic options.

Ureaplasma species

A member of the *Mollicutes* class, *Ureaplasma*, is comprised of two species and 14 serovars. *U. parvum* contains serovars 1, 3, 6, and 14, and *U. urealyticum* contains the remaining serovars (10). All serovars lack cell walls, exhibit limited biosynthetic abilities, hydrolyze urea to generate ATP, and adher to human mucosal surfaces (10). The sequenced *U. parvum* serovar 3 genome is the second smallest known genome with 751, 717 base pairs (12). Genes comprise 93% of the genome with 613 predicted protein-coding genes and 39 RNA-coding genes. About half of the protein-coding genes have been assigned biological functions, 19% of the genes are similar to hypothetical genes of unknown function in other species, and 28% of the genes are unique hypothetical genes with no significant similarities to putative or known genes in other organisms.

Ureaplasma spp. virulence factors

Some serovars have greater association with adverse pregnancy outcomes than others (13–15). Although *U. parvum* is more commonly isolated from clinical adult vaginal and infant respiratory specimens (13,15), and is the predominant species in newborn serum and/or cerebrospinal fluid (CSF) samples detected by PCR (16), Abele-Horn *et al.* (13) reported a higher rate of BPD in *U. urealyticum* respiratory tract colonized infants. In contrast, Katz *et al.* (17) observed no difference in prevalence of either species detected by PCR between infants with and without BPD. To date, there has been no study that has determined the relationship of specific serovars and the development of BPD.

Previously proposed ureaplasmal virulence factors include IgA protease, urease, phospholipases A and C, and production of hydrogen peroxide (12). These factors may allow the organism to evade mucosal immune defenses by degrading IgA, and injuring mucosal cells through the local generation of ammonia, membrane phospholipid degradation and prostaglandin synthesis, and membrane peroxidation, respectively. The ureaplasmal MB antigen that contains both serovar-specific and cross-reactive epitopes, is the predominant antigen recognized during ureaplasmal infections in humans. It exhibits highly variable size *in vitro* and in clinical isolates *in vivo*, suggesting that antigen size variation may be another mechanism through which the organism evades host defenses (10). Although functionally active IgA protease and phospholipase A and C were found in *Ureaplasma* spp., the genes that code for these proteins have not been identified in the *U. parvum* serovar 3 genome (12). The ureaplasmal enzymes may have unique sequences compared with analogous genes in other species.

Association of *Ureaplasma* respiratory tract colonization with chronic lung disease in preterm infants

Because *Ureaplasma* is a commensal in the adult female genital tract, it has been considered of low virulence. However, its presence has been consistently associated with multiple obstetrical complications including infertility, histological chorioamnionitis, stillbirth, preterm delivery, neonatal morbidity, and perinatal death (10,11,18). *Ureaplasma* spp. are the most common organisms isolated from amniotic fluid (AF) and infected placentas (18,19). The vertical transmission rate is inversely related to gestational age (20,21) and increases with duration of rupture of membranes (14). Detection of respiratory tract colonization with *Ureaplasma* by PCR suggests that colonization in very low birth weight

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infants (VLBW <1500g) is higher (25–48%) (10,22) than previously reported for culture-based studies (20%) (7).

The contribution of *Ureaplasma* respiratory tract colonization to the development of BPD has been debated. A meta-analysis of 17 clinical studies published before 1995 supported a significant association between *Ureaplasma* respiratory tract colonization and development of BPD defined as oxygen dependence at 28 to 30 days postnatal age (7), but there were insufficient data concerning the relationship of *Ureaplasma* colonization and BPD at 36 week post-menstrual age (PMA). Most individual studies published since 1995 have supported the association of *Ureaplasma* and BPD (23–28), but other studies failed to show a significant association (29–32). Differences in population characteristics, clinical practices such as antenatal and postnatal steroid use, and culture methodology may account for differences in study conclusions.

Since interpretation of many of the studies published to date has been hampered by inclusion of small numbers of subjects resulting in inadequate power and possible sampling bias, Schelonka *et al*. (8) conducted a meta-analysis of 36 studies published between 1988 and 2004 involving ~3000 preterm infants. Studies were excluded if the proportion of eligible subjects was not described. Included studies were grouped by definition of BPD as oxygen requirement at 28 postnatal days (BPD28; N=23 studies, 2216 subjects) or 36 weeks PMA (BPD36; N=8 studies, 751 subjects). There was a 1.6 (BPD36) to 2.8 (BPD28) -fold increased risk for BPD in *Ureaplasma* colonized infants in the pooled studies. However, substantial heterogeneity was detected, decreasing the precision of the risk estimates. Factors that were related to higher reported odds of an *Ureaplasma*-BPD association included earlier year of publication, small sample size, surfactant use >90%, and endotracheal culture as the only method of diagnosis. Studies published since the last meta-analysis support the *Ureaplasma* respiratory colonization-BPD association (22,33), particularly for the subset of *Ureaplasma*-colonized infants exposed to chorioamnionitis and leukocytosis at birth (33).

The timing and duration of exposure of the developing lung to *Ureaplasma* may be variable and the relationship to pulmonary outcomes is not fully understood. The rate of vertical transmission increases with an increase in duration of rupture of membranes (14), suggesting that for many infants, neonatal infections are the result of ascending infection that occurs at or near parturition. However, *Ureaplasma* species have been detected in AF as early as the time of genetic amniocentesis (16–20 weeks) in 0.36–2.8% (culture-based methods)(34–36) to 12.8% (PCR-based methods) (37) asymptomatic women. While the majority of women in whom early amniotic cavity infection is detected deliver at term (37), those with elevated AF IL-6 levels and midtrimester subclinical *Ureaplasma* intrauterine infection have increased risk for adverse pregnancy outcome including fetal loss and preterm delivery (38). Duration of exposure of the preterm lung postnatally may also be important. Although most epidemiologic studies focused on identifying colonization within the first few days of life, Castro-Alcaraz *et al*. (39) observed that persistent, but not transient, *Ureaplasma* respiratory tract colonization is a risk factor for BPD. Mortality due to respiratory complications is significantly higher in colonized infants (6,21). The risk of a combined outcome measure of BPD or death due to lung disease was 4.2-fold higher in *Ureaplasma* colonized than in non-colonized VLBW infants (22).

Systemic *Ureaplasma* infections in preterm infants

Although the relationship of *Ureaplasma* respiratory tract colonization with BPD has been extensively studied, less is known about the incidence of invasive disease defined as detection in blood and/or CSF, and its relationship with neonatal outcomes. In 2 large

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prospective cohorts, *Ureaplasma* was detected in 17% of cord blood cultures (40) and 23.6% serum and/or CSF PCR samples (16), but invasive disease was not associated with BPD at 36 weeks PMA in either cohort. Overall, almost half of subjects were *Ureaplasma* positive in one or more compartments (respiratory, blood, CSF), confirming that this organism is the most common pathogen affecting this population (16).

Experimental Evidence for Causative Role of *Ureaplasma* spp. in BPD

Elucidating the mechanisms by which *Ureaplasma* may contribute to BPD pathogenesis will not only provide evidence for a causal relationship, but also identify potential targets for interventions to prevent or ameliorate BPD in colonized infants. In this section, we will review our current understanding of the role of inflammation in altered developmental signaling in the preterm lung and the evidence from human studies, and *in vitro* and *in vivo* models that *Ureaplasma*-mediated inflammation produces the BPD phenotype. As shown in Figure 1, we propose that *Ureaplasma* infection initiated *in utero* and augmented postnatally by exposure to volutrauma and oxygen elicits a sustained, dysregulated inflammatory response in the immature lung that impairs alveolarization, and stimulates myofibroblast proliferation, and excessive collagen and elastin deposition.

Chronic inflammation in the immature lung alters developmental signaling and fibrosis

Infection-induced stimulation of inflammatory cytokines may be the causative link between intrauterine infection and neonatal lung injury. AF concentrations of IL-1β, IL-6, TNF-α, and IL-8 were higher in pregnancies producing infants who developed BPD than in pregnancies producing infants without BPD (41). In a series of longitudinal studies comparing temporal changes in inflammatory mediators and their inhibitors in tracheal aspirates from preterm infants, with and without lung disease, we and others have shown an imbalance of pro- and anti-inflammatory cytokines in infants who develop BPD (42–46). The increase in expression of pulmonary pro-inflammatory cytokines, in concert with a decreased capacity to down-regulate this response in infants who develop BPD, suggest that persistent endogenous generation of these cytokines might contribute to chronic lung injury and inflammation.

In transgenic mice, overexpression of TNF-α IL-6, or IL-11 inhibited alveolarization, indicating that prolonged exposure of the preterm lung to a pro-inflammatory environment contributes to abnormal alveolar septation (1). This contention is further supported by Bry and co-workers (47) who developed a bitransgenic CCSP-rtTA-(tetO)-CMV-IL-1β mouse in which IL-1β was expressed under conditional control in airway epithelial cells in the fetal and neonatal lung. IL-1β expression increased from E14.5 until late gestation and decreased postnatally. Postnatal growth was impaired and mortality was higher in the IL-1β expressing newborn mice. The newborn lungs demonstrated many features of the BPD phenotype, including disrupted alveolar septation and capillary development, and disordered α-smooth muscle actin (myofibroblast marker) and elastin deposition in alveolar septa of distal airspaces (47). These abnormalities were attributed to expression of CXC and CC chemokines resulting in recruitment of neutrophils and macrophages to the developing lung. This model demonstrates that inflammation initiated *in utero* early in lung development is sufficient to produce the BPD phenotype.

The effects of prolonged exposure to pro-inflammatory cytokines on alveolarization may be mediated by up-regulation of $TGF\beta_1$. $TGF\beta_1$ plays a role in lung morphogenesis, repair of lung injury, airway remodeling, lung fibrosis, and BPD (48). TGFβ was detected at sites of lung injury in association with myofibroblast proliferation in lungs of infants dying with RDS, implicating TGFβ in the preterm lung response to injury (49). $TGF\beta_1$ overexpression in the lungs of newborn transgenic mice (50) or inoculation with $TGF\beta_1$-expressing

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adenoviral vectors in the newborn rat lung (51) produces a phenotype similar to human BPD with arrested lung saccular and vascular development. TNF-α (52) or IL-1β (53) overexpression in rat lung produces lung fibrosis due to $TGF\beta_1$ stimulation, and induction of myofibroblasts. TNF-α, IL-1β and $TGF\beta_1$ are each elevated in tracheal aspirates of infants who progress to BPD (42,43,54). Taken collectively, these data suggest that prolonged exposure of the developing lung to inflammation contributes to BPD by disrupting normal TGFβ developmental signaling.

Ureaplasma spp modulate the inflammatory response

Recent human and experimental studies confirm that exposure of the fetal and/or newborn lung to *Ureaplasma* contributes to altered lung development, sustained inflammation, and fibrosis. In a review of lung pathology of archived autopsy specimens from *Ureaplasma*-infected preterm infants, we observed moderate to severe fibrosis and increased elastic fiber accumulation (55,56). In addition, we observed increased numbers of myofibroblasts (Fig. 2) and TNF-α and $TGF\beta_1$-immunoreactive cells (Fig. 3) in all *Ureaplasma*-infected infants compared to non-infected gestational controls and infants who died with pneumonia from other causes (55,56). The increase in fibrosis and elastic fiber accumulation in the distal lung correlated spatially and temporally with the presence of $TGF\beta_1$-positive macrophages, suggesting that these are closely linked.

Animal *Ureaplasma* pneumonia models developed in non-human primates and mice demonstrate that an infection established in the pulmonary compartment leads to inflammation and lung injury. Intratracheal *Ureaplasma* inoculation caused an acute bronchiolitis in 140 d preterm baboons (57), and an acute interstitial pneumonia in newborn, but not 14d old mice (58). Hyperoxia exposure increased mortality, lung inflammation, and delayed pathogen clearance in *Ureaplasma*-inoculated newborn mice (59), consistent with the hypothesis that *Ureaplasma* augments the inflammatory response to secondary stimuli. In mice, *Ureaplasma* intratracheal inoculation caused an acute pneumonitis, sustained inflammation up to 28 d post-inoculation despite apparent clearance of the organism (60). Although these models demonstrate the direct effect of *Ureaplasma* in the lung, they lack the early developmental component.

Intrauterine *Ureaplasma* infection models have been developed in non-human primates (61–64) and in fetal sheep (65,66) that more closely mimic the human condition. In Rhesus monkeys, intraamniotic inoculation of *U. parvum* serovar 1 at 130 d gestation (term, 167 d) increased uterine contractility, preceded by elevations in TNF-α, IL-1β, IL-6, and IL-8 in AF and histological evidence of chorioamnionitis (61,62). Similar findings were observed in the 125 d immature baboon model infected with *Ureaplasma in utero* (63). Intra-amniotic *Ureaplasma* (serovar 1) inoculation 2 d prior to delivery at 125 d (67% of term gestation) in the baboon caused an inflammatory response in the amniotic and fetal lung compartments and vertical transmission to the fetal lung that persisted up to 2 weeks postnatally in half of the antenatal-exposed animals. Compared to lungs from non-infected animals and gestational controls, *Ureaplasma*-infected lungs demonstrated 1) more extensive fibrosis, increased myofibroblast phenotype (Fig. 4) and $TGF\beta_1$ immunostaining (Fig. 5); Fig. 2) increased bronchoalveolar lavage concentrations of IL-1β, and active TGF β_1, but no differences in IL-10; and 3) a trend towards greater activation of pro-fibrotic transcription factors Smad-2 and -3 relative to anti-fibrotic Smad-7 in lung homogenates, suggesting an imbalance of pro- and anti-fibrotic signaling factors in the *Ureaplasma*-infected animals. In fetal sheep exposed to intra-amniotic *Ureaplasma* for periods up to 10 wks, long-term exposure was associated with improvement in lung function, but poor fetal growth, fetal acidemia, and evidence of fetal pulmonary inflammation (65). Intra-amniotic inoculation of *U. parvum* servovar 3 or 6 at mid-gestation in fetal sheep did not result in preterm labor, but did cause placental and fetal pulmonary inflammation and altered lung development whether

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delivery occurred preterm or at term (66). These data provide compelling evidence that antenatal *Ureaplasma* infection alters lung development and augments a prolonged, pro-inflammatory, pro-fibrotic response in the preterm lung exposed postnatally to ventilation and hyperoxia.

We propose that *Ureaplasma* may contribute to lung injury and fibrosis by modulating the local immune response to produce sustained chronic inflammation. Preterm infants with *Ureaplasma* respiratory colonization exhibited elevated tracheal aspirate IL-1β, TNF-α, and monocyte chemoattractant protein-1 concentrations and neutrophil chemotactic activity during the first weeks of life compared to non-colonized infants (67–69). In the mouse *Ureaplasma* pneumonia model, intratracheal inoculation with *Ureaplasma* induced a prolonged inflammatory response as indicated by a sustained recruitment of neutrophils and macrophages into the lung (60).

The stimulatory effect of *Ureaplasma* on cytokine release has been confirmed *in vitro*. In cultured human cord blood preterm monocytes, *Ureaplasma* stimulated release of TNF-α and IL-8, and when co-administered with Gram-negative lipopolysaccharide (LPS), *Ureaplasma* greatly augmented generation of pro-inflammatory cytokines while blocking expression of the counter-regulatory cytokines, IL-6 and IL-10 (70). *Ureaplasma* stimulated TNF-α and IL-6 release, nitric oxide production, and up-regulation of iNOS, nuclear factor-kappa B (NF-κB) activation, and VEGF and soluble and cell-associated ICAM-1 expression by human and murine derived monocytic cells (71–73). *Ureaplasma* induced apoptosis in A549 cells, a human type II cell line, and in THP-1 human monocytic cells (74). These effects could be partially blocked by anti-TNF-α monoclonal antibody (73,74), implicating TNF-α as a mediator of the host immune response to this infection that contributes to altered lung development.

Ureaplasma TLR signaling

The Toll like receptors (TLRs) are “pattern recognition receptors” that are key components of the innate immune response to microbial products (75). The TLR family responds to a broad range of pathogen-associated molecular patterns (PAMPs), including LPS, viral coat proteins, bacterial lipoproteins and glycolipids, viral RNA, and CpG-containing bacterial DNA (75). Engagement of TLR proteins activates the expression of pro-inflammatory mediators by macrophages, neutrophils, dendritic cells, B cells, endothelial cells, and epithelial cells.

Recent studies by Peltier *et al.* (76) and Shimizu *et al.* (77) demonstrated that Triton X-114 detergent extracted lipoproteins from *U. urealyticum* serovar 4 and *U. parvum* serovar 3, respectively, are responsible for NF-κB activation. Active lipoproteins identified for serovar 3 included the MB antigen (77). The serovar 3 detergent extracts activated NF-κB through TLR2 cooperatively with TLR1 and TLR6 (77), while serovar 4 extracts activated both TLR2 and TLR4 (76). Further studies will need to determine if the different *Ureaplasma* species or serovars interact with different TLRs.

Little is known concerning TLR expression during human lung development. In mice, TLR2 and TLR4 mRNA levels were barely detectable early in gestation, increasing thereafter during late gestation and postnatally (78). In fetal sheep lung, TLR2 and TLR4 mRNA levels increased throughout late gestation to reach half of adult levels at term, but were induced by intra-amniotic LPS exposure (79). In the immature baboon model, TLR2 and TLR4 mRNA and protein expression were low in125d and 140d non-ventilated gestational controls, reached adult levels near term, and were increased in 125d preterm baboons ventilated with oxygen for 21 days (80). These data may explain, in part, the developmental susceptibility to *Ureaplasma* infection and interaction with other stimuli. Low TLR2 and 4

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expression early in gestation may increase the susceptibility of the fetal lung to *Ureaplasma* infection and delay clearance, but postnatal exposures to mechanical ventilation, oxygen, and other infections, may stimulate pulmonary TLR expression and enhance *Ureaplasma*-mediated inflammatory signaling.

Therapeutic Considerations

Despite *in vitro* susceptibility of *Ureaplasma* to erythromycin, trials of erythromycin therapy in the first few weeks of life in *Ureaplasma* colonized preterm infants failed to demonstrated efficacy to prevent BPD or eradicate respiratory tract colonization (reviewed in (10)). Combined antibiotic treatment with ceftriaxone, clindamycin, and erythromycin failed to eradicate mycoplasma invasion of the amniotic cavity or resolve inflammation in most patients with preterm premature rupture of membranes (81). Lack of efficacy may have been due to the late timing or choice of antibiotic(s) or lack of combination with anti-inflammatory drugs. In an experimental *Ureaplasma* intraamniotic infection (IAI) in Rhesus monkeys, azithromycin alone or in combination with dexamethasone and indocin prevented fetal lung damage (Novy, MJ *et al*., Maternal azithromycin (AZI) therapy for *Ureaplasma* intraamnioitc infection (IAI) prevents advanced fetal lung lesions in rhesus monkeys, 2008 SGI Annual Scientific Meeting, March 26–29, 2008, San Diego, CA, Abstract 438).

Eradicating *Ureaplasma* spp. from the pregnant female genital tract and/or fetal/newborn lung may be difficult. Clinical isolates may vary in susceptibility to macrolide antibiotics due to 1) mutations in 23S rRNA (82); 2) presence of co-infection with *Mycoplasma hominis* (83); and 3) differences in ability to form protective biofilms (84). The pharmacokinetics, pharmacodynamics, and safety of macrolides in newborns are unknown and would need to be established before a randomized placebo-controlled trial of efficacy of these antibiotics to eradicate *Ureaplasma* infection or prevent BPD in the preterm population can be done.

Studies of the *Ureaplasma* genome and insight into the pathogen-host interactions may identify alternative drug and vaccine targets and lead to the development of biomarkers for early detection of infection. If essential membrane transporters lacking significant homology with human proteins are identified in the *Ureaplasma* spp. genomes, they might be attractive therapeutic targets for small molecule inhibitors or vaccines (85). The TLR activating lipoproteins are additional attractive targets for drug and/or vaccine development. Since most *Ureaplasma* IAIs are subclinical, there are currently no effective strategies for screening for affected pregnancies. Proteomic analysis of AF (61) and cervical-vaginal fluid (62) in experimental Rhesus monkey *Ureaplasma* intraamniotic infection revealed unique proteins that may be useful as specific biomarkers for detection of early intraamniotic infection. Future studies will need to focus on strategies for prevention and early detection of *Ureaplasma* intraamniotic infection, and the development of optimal antibiotic therapy for treating the infection *in utero* to reduce preterm birth and in the preterm newborn to prevent BPD.

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ABBREVIATIONS

AF	Amniotic fluid
BPD	bronchopulmonary dysplasia

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CSF	cerebrospinal fluid
IAI	intraamniotic infection
LPS	lipopolysaccharide
NF-κB	nuclear factor-kappa B
PMA	post-menstrual age
TLR	Toll-like receptor

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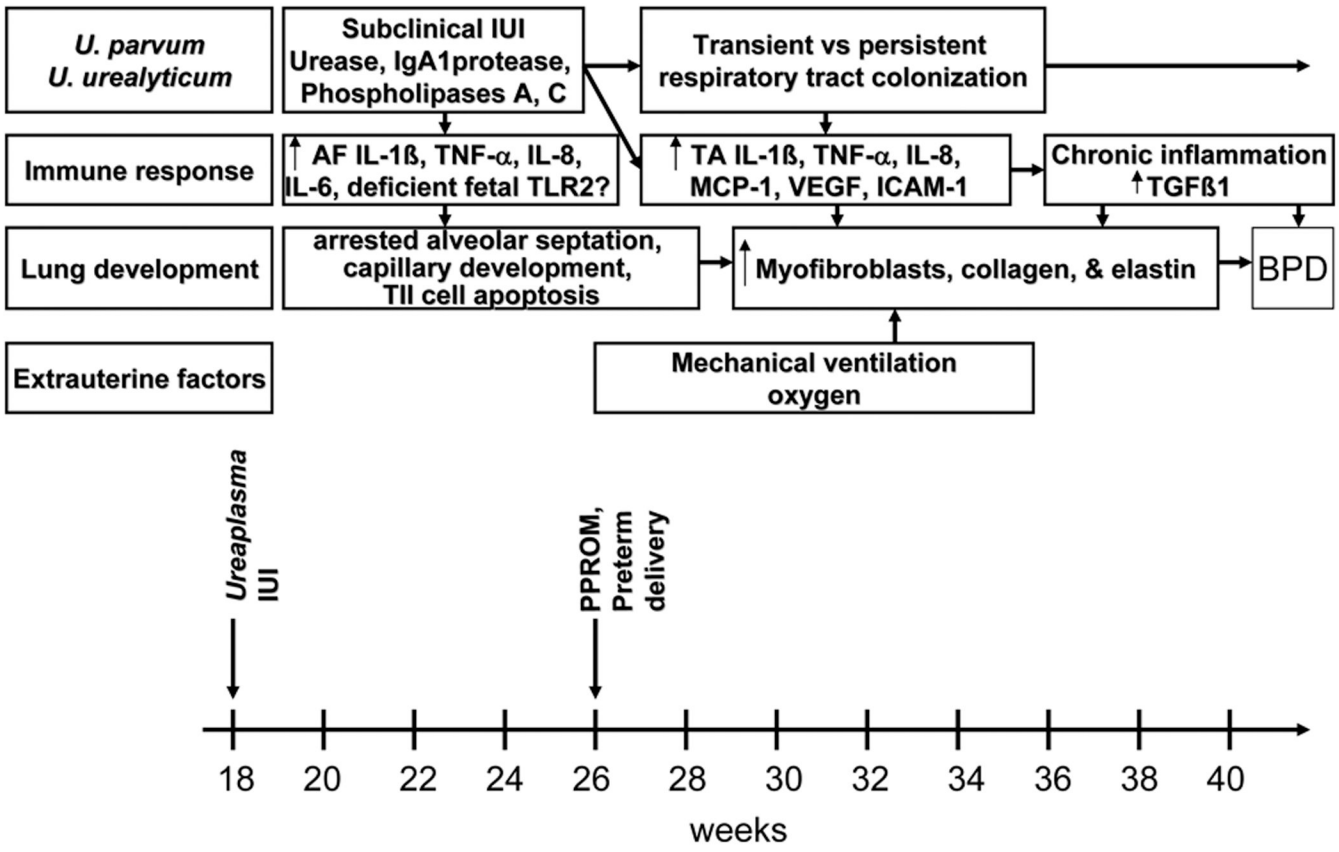

Figure 1. Proposed model for role of *Ureaplasma* infection in BPD pathogenesis

In this schematic, prolonged intraamniotic exposure of the fetal lung to *Ureaplasma* infection and maternal and fetal derived cytokines, recruits inflammatory cells, and alters TGFβ1 developmental signaling in the lung. Postnatal exposure to ventilation and oxygen augments this pro-inflammatory response leading to arrested alveolarization, disordered myofibroblast proliferation, and excessive collagen and elastin deposition.

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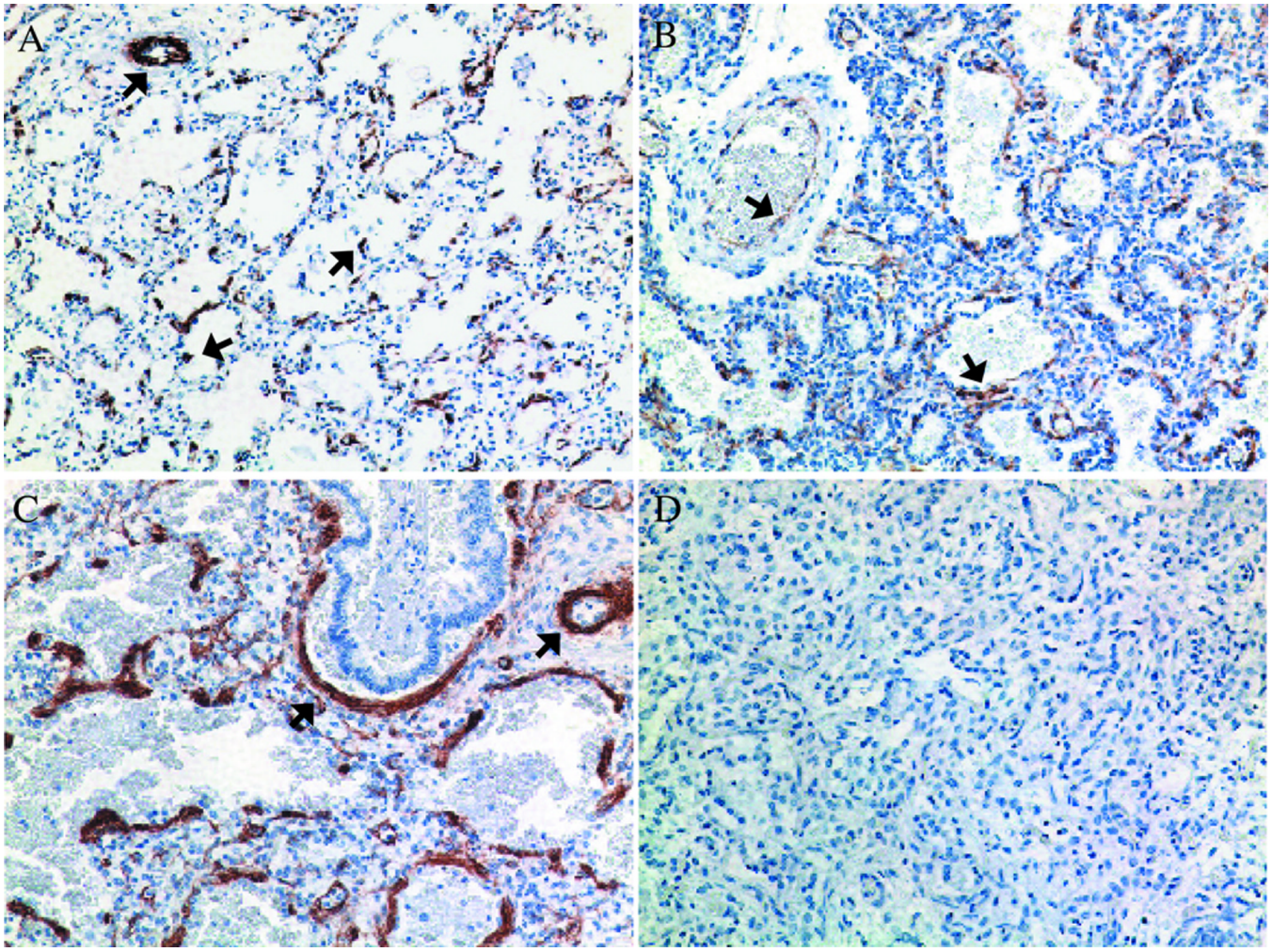

Figure 2. Comparison of α-smooth muscle actin (α-SMA) immunostaining in human preterm lung specimens

(A) Control non-ventilated infant at 23 wk GA; (B) Other pneumonia case at 24 wk GA ventilated for 2 d; and (C) *Ureaplasma*-infected infant at 26 wk GA ventilated for 20 d. Lung sections were incubated with anti-αSMA antibody and counterstained with hematoxylin. Negative controls were processed in the absence of primary antibody (D) (Magnification 200x). αSMA immunoreactive cells were noted surrounding vessel walls (arrows) in (A) and distributed in a pattern of thickened clusters of cells often surrounding terminal airways in other pneumonia and *Ureaplasma* cases (B and C). The extent of myofibroblast accumulation and percent of lung involvement was greater in *Ureaplasma* cases than in other pneumonia cases. Reprinted from Viscardi *et al.* Pediatr Dev Pathol 9:143–151, Copyright © 2006 Society for Pediatric Pathology and the Paediatric Pathology Society, with permission.

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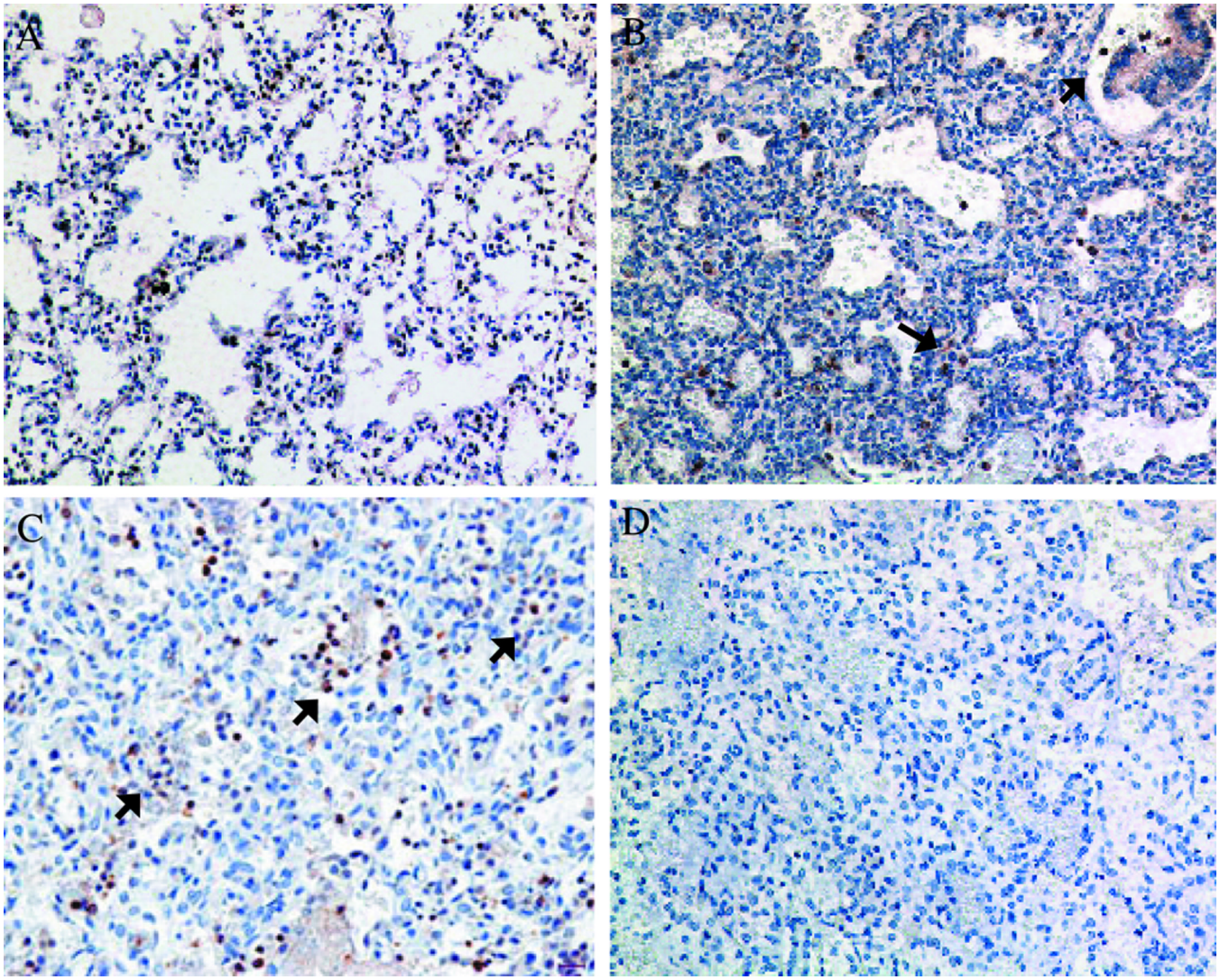

Figure 3. Comparison of TGFβ$_1$ immunostaining in human lung specimens

(A) Control non-ventilated infant at 23 wk GA; (B) Other pneumonia case at 24 wk GA ventilated for 2 d; and (C) *Ureaplasma*-infected infant at 26 wk GA ventilated for 20 d. Lung sections were incubated with anti-TGFβ$_1$ antibody, stained with diaminobenzidine and counterstained with hematoxylin. Negative controls were processed in the absence of primary antibody (D) (Magnification 200x). In lung specimens from infants dying with acute bacterial or *Ureaplasma* pneumonia, immunostaining was concentrated in focal aggregates of alveolar and interstitial macrophages. Reprinted from Viscardi *et al.* Pediatr Dev Pathol 9:143–151, Copyright © 2006 Society for Pediatric Pathology and the Paediatric Pathology Society, with permission.

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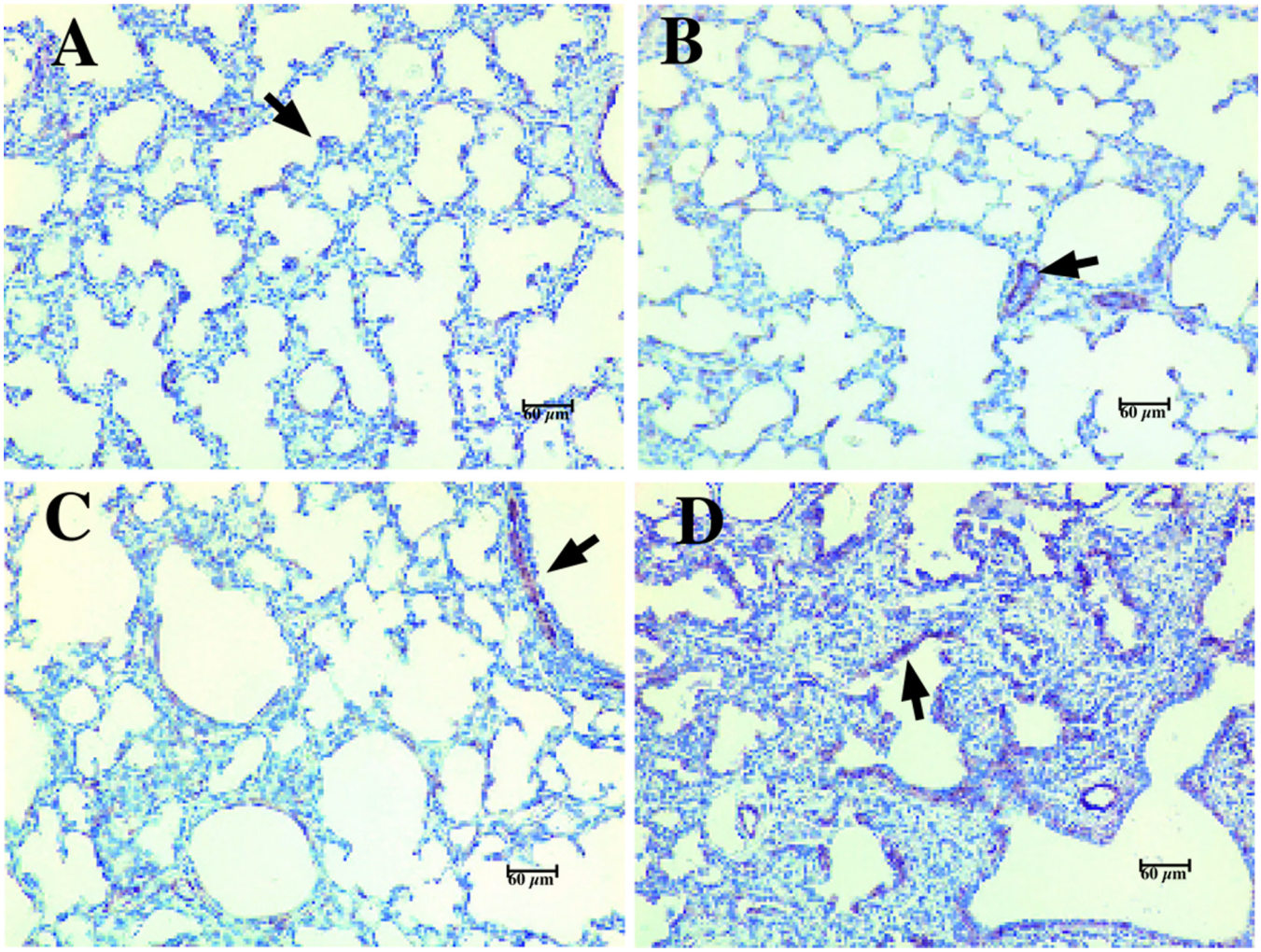

Figure 4. Alpha-SMA positive cells are increased in lung tissue form antenatal Ureaplasma-infected baboons

Lung sections were processed for immunohistochemical staining using a monoclonal anti-human antibody directed against α-SMA (arrows). A) 125d GC; B) 140d GC; C) 125d non-infected ventilated control; and D) 125d antenatal *Ureaplasma*-infected, ventilated baboon. Magnification 200X. Reprinted from Viscardi *et al.* Pediatr Res 60:141–146, Copyright © 2006 International Pediatric Research Foundation, Inc., with permission.

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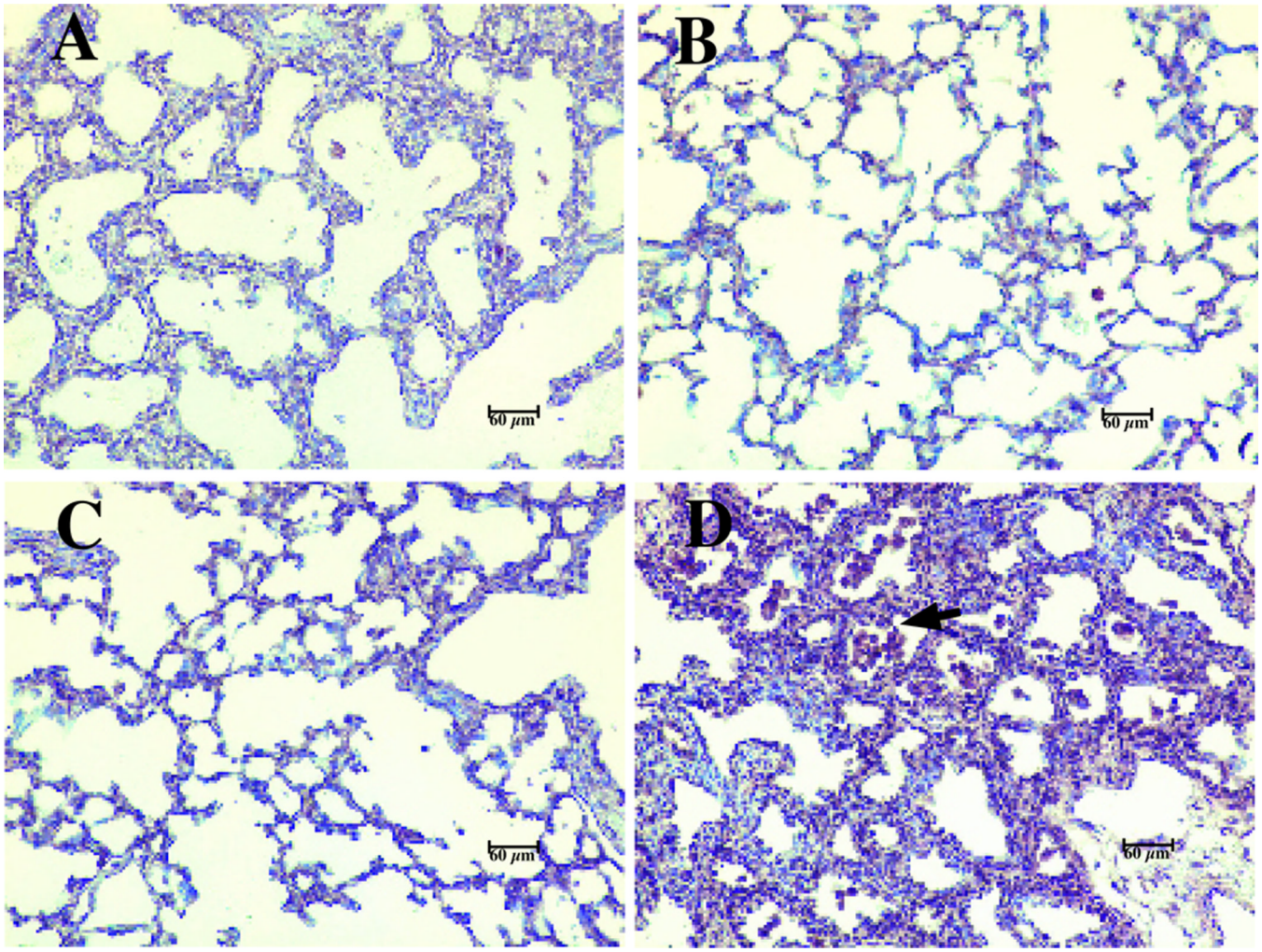

Figure 5. TGFβ$_1$ immunostaining in lung specimens of antenatal *Ureaplasma*-exposed baboons is concentrated in focal aggregates of alveolar and interstitial macrophages

Lung sections were incubated with anti-TGFβ$_1$ antibody, stained with diaminobenzidine and counterstained with hematoxylin. A) 125d GC; B) 140d GC; C) 125d non-infected ventilated control; and D) 125d antenatal *Ureaplasma*-infected, ventilated baboon. Magnification 200X. Reprinted from Viscardi *et al.* Pediatr Res 60:141–146, Copyright © 2006 International Pediatric Research Foundation, Inc., with permission.