Inflammation in fetal sheep from intra-amniotic injection of *Ureaplasma parvum*

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—Bronchopulmonary dysplasia is associated with chorioamnionitis and fetal lung inflammation. *Ureaplasma* species are the bacteria most frequently isolated from chorioamnionitis. Very chronic ureaplasma colonization of amniotic fluid causes low-grade lung inflammation and functional lung maturation in fetal sheep. Less is known about shorter exposures of the fetal lung. Therefore, we hypothesized that ureaplasmas would cause an acute inflammatory response that would alter lung development. Singleton ovine fetuses received intra-amniotic *U. parvum* serovar 3 or control media at 110, 117, or 121 days and were delivered at 124 days gestational age (term = 150 days). Inflammation was assessed by 1) cell counts in bronchoalveolar lavage fluid (BALF), and 2) cytokine mRNA measurements, immunohistochemistry, and flow cytometry for inflammatory cells and elastin and α-smooth muscle actin (α-SMA) staining in lung tissue. Neutrophils were increased in BALF 3 days after exposure to ureaplasmas (P = 0.01). Myeloperoxidase-positive cells increased after 3 days (P = 0.03), and major histocompatibility complex (MHC) class II-positive cells increased after 14 days of ureaplasma exposure (P = 0.001). PU.1 (macrophage marker)- or CD3 (T lymphocyte marker)-positive cells were not induced by ureaplasmas. CD3-positive cells in the posterior mediastinal lymph node increased in ureaplasma-exposed animals at 3, 7, and 14 days (P = 0.002). Focal elastin depositions decreased in alveolar septa at 14 days (P = 0.002), whereas α-SMA increased in arteries and bronchioli. *U. parvum* induced a mild acute inflammatory response and changed elastin and α-SMA deposition in the lung, which may affect lung structure and subsequent development.

inflammatory response; elastin; ureaplasma; lung development

CHORIOAMNIONITIS THAT IS OFTEN clinically asymptomatic is associated with the majority of early gestational preterm births (2, 12). The fetus is born with inflamed lungs and often a systemic inflammatory response (10, 53). This fetal lung inflammation is associated with the development of bronchopulmonary dysplasia (BPD) (44, 47). In experimental models, LPS and intra-amniotic and/or maternal IL-1 can modulate lung development/maturity (8, 23, 29, 38, 54, 57), but the major organisms associated with chorioamnionitis are *Ureaplasma* species, which are very small bacteria without a cell wall (45, 59). Ureaplasmas are chronically tolerated in the urogenital tracts of men and women and can be present in amniotic fluid (AF) without inducing preterm labor (16, 41). Chronic fetal exposure to *U. parvum* induced functional lung maturation in fetal lambs (35–37) but did not change lung function or modulate the lung injury and inflammation caused by high tidal volume ventilation (43). Very short-term intratracheal exposures to ureaplasmas caused pulmonary inflammatory responses in fetal mice (39), baboons (51, 58), and rhesus macaque monkeys (40). Short-interval fetal exposures to ureaplasmas with subsequent ventilation for 14 days after preterm birth initiated fibrosis and altered developmental signaling in the lungs of preterm baboons (51), indicating that chronic postnatal ventilation aggravated the lung injury. It is, however, not clear when these structural changes occurred or whether ureaplasma exposure in utero alone was sufficient to induce developmental changes in the short term.

Given the chronic nature of ureaplasma infections, we hypothesized that *U. parvum* would induce an initial acute fetal lung inflammatory response that would lead to changes in lung development. Pregnant ewes were given *U. parvum* serovar 3 by intra-amniotic injection 3, 7, or 14 days before preterm delivery at 124 days gestational age (GA). We evaluated inflammation in the lung and its draining lymph node, the posterior mediastinal lymph node (PMLN). Inflammatory cells were identified by histological and immunohistochemical analysis using CD3 for T cells (11), myeloperoxidase (MPO) for neutrophils, and PU.1, a transcription factor that is highly expressed in differentiated alveolar macrophages (11, 27). To evaluate whether changes in lung development had occurred as a result of exposure to *U. parvum*, we measured markers of lung damage and assessed changes in the structural proteins elastin and α-smooth muscle actin (α-SMA) that are involved in alveolar septation (4, 9, 25).

MATERIALS AND METHODS

Intra-amniotic injections. All studies were approved by the Animal Ethics Committees at the University of Western Australia, the Queensland University of Technology, and the Cincinnati Children’s Hospital Medical Center. Ureaplasmas were grown and prepared for the ultrasound-guided injections as reported previously (35). Time-mated ewes with singleton fetuses were randomized to receive either an intra-amniotic injection of *U. parvum* serovar 3 [2 × 10⁷](http://ajplung.physiology.org) by 10.220.33.5 on April 3, 2017 Downloaded from

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The PMLN was fixed in 10% buffered formalin. The right, upper lobe used for total and differential cell counts and protein measurements. Resultant bronchoalveolar lavage fluid (BALF) from the left lung was and the left lung was lavaged three times with 0.9% NaCl (18). The no. MCA2220B; AbD Serotec). After a washing step to remove labeled from the cell suspension with anti-CD45 biotin antibodies (cat. no. S203110X; Miltenyi Biotec). CD45-positive cells were isolated by magnetism. CD45-positive cells added, and the suspension was passed through MACS columns for separation of CD45-positive cells by magnetism. CD45-positive cells were counted and incubated with monoclonal anti-major histocompatibility complex (MHC) class II antibody (cat. no. H42A; Veterinary Medical Research and Development (VMRD)). An IgG2a isotype antibody (cat. no. sc-3878; Santa Cruz Biotechnology) was used as a negative control. MHC class II-positive cells labeled with an anti-IgG2a-RPE secondary antibody (cat. no. 1080-09; Southern Biotechnology Associates) were counted by flow cytometry (BD FACSCantoII and BD FACSDiva software; Becton Dickinson).

**Immunohistochemistry.** Paraffin-embedded RULL sections (4 μm, transverse) were stained for CD3 (cat. no. A0452; Dako), MPO (cat. no. A0398; Dako), PU.1 (cat. no. sc-352; Santa Cruz Biotechnology), and α-SMA (cat. no. A5228; Sigma-Aldrich) (20). Sections of the PMLN (4 μm, transverse) were stained for CD3 only. Briefly, the sections were deparaffinized with xylol, 100% ethanol, 96% ethanol, and 70% ethanol. Subsequently, endogenous peroxidase activity was blocked by incubation with 0.3 or 0.5% hydrogen peroxide. Antigen retrieval was performed by incubating the slides in heated citrate buffer (cat. no. S203110X; REAL Target Retrieval Solution 10X; Dako) for 30 min. To block nonspecific binding, the tissue sections were incubated with either 20% normal goat serum (NGS) or 5% BSA/PBS in a humidified box for 30 min (this step was omitted for α-SMA). After adding the primary antibody, the sections were incubated overnight at 4°C. A negative control was included by incubating with 0.1% BSA/PBS (for CD3, MPO, and PU.1) or 2% NGS/PBS (for α-SMA) instead of the primary antibody. After incubation with polyclonal swine anti-rabbit biotin-labeled secondary antibody (cat. no. E0353; Dako), the sections were incubated with StrepABComplex/HRP (cat. no. K0377; Dako). The sections were then washed with 0.1 M acetate buffer and incubated for 4 min with nickel sulfate-diaminobenzidine (NiDAB) solution in a closed, dark box. Subsequently, the tissue sections were rinsed with Tris-saline and incubated with Tris-cobalt. After counterstaining with 0.1% nuclear fast red, the sections were washed and finally dehydrated in a series of 70, 96, and 100% ethanol, followed by xylol. Evaluation was performed by light microscopy (Axioplan 40; Zeiss) with Leica QWin software (Leica Microsystems, Mannheim, Germany). Sections were scored for positive CD3, MPO, and PU.1 staining with a semiquantitative scoring system: 1, little staining; 2, some staining; and 3, heavy staining. The intensity of staining was measured as described previously (37).

**FACS analysis.** Single cell suspensions of inflammatory cells were recovered from tissue of the right lung (22) using the MACS Cell Separation system (Miltenyi Biotec). CD45-positive cells were isolated from the cell suspension with anti-CD45 biotin antibodies (cat. no. MCA2220B; AbD Serotec). After a washing step to remove unbound antibody, MACS anti-biotin magnetic microbeads were added, and the suspension was passed through MACS columns for separation of CD45-positive cells by magnetism. CD45-positive cells were counted and incubated with monoclonal anti-major histocompatibility complex (MHC) class II antibody (cat. no. H42A; Veterinary Medical Research and Development (VMRD)). An IgG2a isotype antibody (cat. no. sc-3878; Santa Cruz Biotechnology) was used as a negative control. MHC class II-positive cells labeled with an anti-IgG2a-RPE secondary antibody (cat. no. 1080-09; Southern Biotechnology Associates) were counted by flow cytometry (BD FACSCantoII and BD FACSDiva software; Becton Dickinson).

**Table 1. Observations of fetal lambs (GA 124 days) exposed to Ureaplasma parvum**

<table>
<thead>
<tr>
<th>Assessments at delivery</th>
<th>Control, n = 8</th>
<th>UP 3-Day, n = 7</th>
<th>UP 7-Day, n = 6</th>
<th>UP 14-Day, n = 5</th>
</tr>
</thead>
<tbody>
<tr>
<td>Body wt, kg</td>
<td>2.7 ± 0.1</td>
<td>2.7 ± 0.1</td>
<td>2.8 ± 0.1</td>
<td>2.9 ± 0.1</td>
</tr>
<tr>
<td>Cord blood, pH</td>
<td>7.1 ± 0.03</td>
<td>7.2 ± 0.03</td>
<td>7.2 ± 0.03</td>
<td>7.2 ± 0.03</td>
</tr>
<tr>
<td>White blood cell counts in cord blood, × 10⁶ cells/l</td>
<td></td>
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</tr>
<tr>
<td>Lymphocytes</td>
<td>2.3 ± 0.2</td>
<td>2.6 ± 1.0</td>
<td>2.9 ± 0.2</td>
<td>2.9 ± 0.4</td>
</tr>
<tr>
<td>Neutrophils</td>
<td>0.6 ± 0.1</td>
<td>0.4 ± 0.1</td>
<td>0.5 ± 0.02</td>
<td>0.7 ± 0.1</td>
</tr>
<tr>
<td>Monocytes</td>
<td>0.2 ± 0.03</td>
<td>0.2 ± 0.1</td>
<td>0.2 ± 0.1</td>
<td>0.1 ± 0.02</td>
</tr>
<tr>
<td>Cytokine and surfactant protein mRNA levels in the lung</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>IL-1β</td>
<td>1.0 ± 0.1</td>
<td>6.7 ± 3.8</td>
<td>0.8 ± 0.3</td>
<td>2.0 ± 0.5</td>
</tr>
<tr>
<td>IL-6</td>
<td>1.0 ± 0.1</td>
<td>5.5 ± 3.3</td>
<td>0.8 ± 0.3</td>
<td>1.2 ± 0.1</td>
</tr>
<tr>
<td>SP-A</td>
<td>1.0 ± 0.2</td>
<td>1.9 ± 1.0</td>
<td>3.2 ± 2.2</td>
<td>1.4 ± 0.6</td>
</tr>
<tr>
<td>SP-B</td>
<td>1.0 ± 0.1</td>
<td>1.3 ± 0.6</td>
<td>1.9 ± 0.9</td>
<td>1.5 ± 0.4</td>
</tr>
<tr>
<td>Total protein, μg/ml BALF</td>
<td>245 ± 26</td>
<td>319 ± 57</td>
<td>332 ± 87</td>
<td>246 ± 64</td>
</tr>
<tr>
<td>V40, ml/kg body wt</td>
<td>12.2 ± 2.0</td>
<td>6.2 ± 0.6</td>
<td>19.3 ± 4.0</td>
<td>14.5 ± 4.3</td>
</tr>
</tbody>
</table>

Data are expressed as means ± SE. GA, gestational age; UP, U. parvum; SP, surfactant protein; BALF, bronchoalveolar lavage fluid; V40, lung volume at 40 cmH2O. *P < 0.05 vs. controls using 1-way ANOVA with Dunnett post hoc test or a nonparametric Kruskal-Wallis ANOVA with Dunn test for post hoc analysis as appropriate.

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Immunofluorescence. U. parvum serovar 3 staining was performed on paraffin-embedded RULL sections (4 μm, transverse) using a protocol modified from our previous study (35). Aspecific binding was blocked using 20% NGS/PBS before overnight incubation at 4°C with polyclonal rabbit anti-U. parvum serovar 3 antibody. After incubation with polyclonal swine anti-rabbit biotin-labeled secondary antibody (cat. no. E0353; Dako) for 1 h, the sections were incubated with fluorescent quantum dots (cat. no. Q10111MP, Qdot 585, streptavidin conjugate; Invitrogen) for 30 min at room temperature in a closed, dark box. Subsequently, sections were washed in PBS and mounted in glycerol/1,4-diazabicyclo(2.2.2)octane (DABCO) anti-fade/4',6'-diamidino-2-phenylindole (DAPI). Imaging was performed with two-photon microscopy (Leica TCS SP5 system) using a Compact Ultrafast Ti:Sapphire Laser (Chameleon; Coherent) for excitation.

Fig. 2. U. parvum serovar 3 in the lung. Fluorescent immunohistochemistry performed on lung tissue from fetal lambs exposed to UP for 3 days (A) and media (B). UP were detected by culture in all UP-exposed lungs and not in media-exposed lungs. Lung tissue is depicted in green, and UP in red. The high magnification inset in A depicts UP inside the alveolar space.

Fig. 3. Inflammatory cell numbers in bronchoalveolar lavage fluid (BALF), myeloperoxidase (MPO), transcription factor PU.1, and major histocompatibility complex class II-positive (MHCII+) cells in the lung. A: the number of neutrophils was increased in the BALF of UP 3-day fetuses compared with controls. *P < 0.05 vs. controls using a Kruskal-Wallis ANOVA. B: the number of monocytes did not change significantly in the BALF of UP-exposed fetuses. C: MPO-positive cells increased in lung tissue after 3 days of UP exposure compared with controls. *P < 0.05 vs. controls using a 1-way ANOVA with Dunnett post hoc test. D: the PU.1-positive cells in lung tissue exposed to UP did not differ significantly from controls. E: MHCII+ cells increased in the lungs of UP 14-day animals. *P < 0.05 vs. controls using a Mann-Whitney test.
at a wavelength of 840 nm and Leica Application Suite Advanced Fluorescence imaging software (Leica Microsystems).

**Elastin staining.** Elastin staining was performed on RULL sections (4 μm, transverse) (25). Briefly, the sections were deparaffinized in an ethanol series and incubated for 20 min in Weigert resorcin-fuchsin (cat. no. 2E 030; Chroma) at 60–70°C. After rinsing with water, the sections were incubated for 3 min in a tartrazine solution at room temperature. Subsequently, the sections were washed and dehydrated in ethanol and xylol. Elastin foci were counted using ImageJ software.

**Surfactant protein and cytokine mRNA.** Surfactant protein A, B, and C mRNA levels and proinflammatory cytokine mRNAs for IL-1β and IL-6 were measured by RNase protection analysis (24) using RNA from the right, lower lobe. Briefly, solution hybridization was performed for 16 h using a molar excess of [α-32P]UTP-labeled riboprobes. Unhybridized, single-strand RNA was digested with RNase A/T1 (PharMingen, San Diego, CA). RNase was then inactivated, and protected RNA was precipitated using the RPA III inactivation buffer (Ambion, Austin, TX). The ribosomal protein mRNA L32 was used as an internal control. The protected fragments were resolved on 6% polyacrylamide 8 mol/l urea gels, visualized by autoradiography, and quantified on a PhosphorImager using ImageQuant version 1.2 software (Molecular Dynamics, Sunnyvale, CA). Results from the control group were standardized to 1, and results from ureaplasma-exposed groups were expressed as fold changes.

**Measurement of total protein.** Total protein in the BALF was measured using the Lowry assay (31).

**Data analysis.** Results are given as means ± SE. The groups were compared using one-way ANOVA with Dunnett test for post hoc analysis or a nonparametric Kruskal-Wallis ANOVA with Dunn test for post hoc analysis as appropriate. Two-group comparisons were done by Mann-Whitney test. Statistical analysis was performed by GraphPad Prism version 5.0. Significance was accepted at P < 0.05.

**RESULTS**

**Fetuses exposed to U. parvum serovar 3 and media control.** Of the 28 animals randomized, there were 1 stillbirth in the control group and 1 malformed and growth-restricted fetus in the 14-day ureaplasma exposure group; both animals were excluded from the analysis. Body weight and cord blood pH values were not altered in fetuses exposed to ureaplasmas compared with controls (Table 1). The lung gas volume (V40), measured at a pressure of 40 cmH2O, showed a trend for decrease in fetuses delivered 3 days after exposure to ureaplasmas compared with controls, but this was not significant.

**Culture and fluorescent immunohistochemistry for U. parvum.** Cultures of AF and lung tissue of animals exposed to ureaplasmas were all positive, with $10^4$–$10^6$ CFU/ml in AF. The lung tissues contained ureaplasmas at average titers of $2.3 \times 10^6$ CFU/g at 3 days, $5.3 \times 10^5$ CFU/g at 7 days, and $2.0 \times 10^5$ CFU/g at 14 days after exposure.

Fig. 4. CD3-positive cells in the fetal ovine lung after exposure to U. parvum serovar 3. Expression of CD3 in the lung was evaluated in controls (A) and after exposure to UP for 3 days (B), 7 days (C), or 14 days (D) before delivery at 124 days GA. E: there were no changes in CD3-positive cells as a result of exposure to UP. P < 0.05 vs. controls using a 1-way ANOVA with Dunnett post hoc test.
1.7 × 10⁶ CFU/g at 14 days of exposure. No ureaplasmas were detected in the AF or lungs of fetuses exposed to media. Immunohistochemistry for U. parvum in the fetal lung demonstrated organisms in the alveoli of the ureaplasma-exposed animals (Fig. 2A). No ureaplasmas were found in lung sections of media-exposed animals (Fig. 2B).

Systemic and local inflammatory mediators. Fetal systemic inflammation was assessed by the number of lymphocytes, neutrophils, and monocytes in the cord blood. There were no changes in the levels of systemic inflammatory cells in ureaplasma-exposed fetuses compared with controls (Table 1).

Inflammation in the lung was assessed by the number of neutrophils, monocytes, and lymphocytes in the BALF. The absolute BALF neutrophil count increased after 3 days of exposure (Fig. 3A). The number of monocytes did not change significantly in the BALF of ureaplasma-exposed fetuses (Fig. 3B). In most BALF samples, no lymphocytes were counted in either control or experimental groups (data not shown). Therefore, ureaplasmas induced a modest neutrophil response. Markers for inflammatory cells were used to assess the inflammatory cells in lung tissue. MPO-positive cells were increased in the ureaplasma 3-day fetuses, but this increase was not consistent in the lung tissue of ureaplasma 7- and 14-day fetuses (Fig. 3C). Cells expressing PU.1, a macrophage-specific transcription factor, did not increase in the lung tissue (Fig. 3D). The percentage of MHC class II-positive cells in the CD45 cell population increased after 14 days of ureaplasma exposure, as measured by flow cytometry (Fig. 3E). This analysis was not performed for ureaplasma 3- or 7-day animals. Ureaplasma exposure did not change mRNA levels of the proinflammatory cytokines IL-1β and IL-6 in the lung nor did it increase the mRNA levels of surfactant proteins A, B, and C (Table 1). Furthermore, there was no increase of total protein levels in the BALF (Table 1).

CD3-positive cells were not increased in the lungs of animals exposed to ureaplasmas (Fig. 4). However, compared with media-exposed fetuses (Fig. 5A), CD3-positive cells increased in the PMLN of the fetuses that had been exposed to ureaplasmas for 3 days (Fig. 5B), 7 days (Fig. 5C), or 14 days (Fig. 5D). Figure 5E shows the increases between the different experimental groups. There was a tendency for increased PMLN weight after exposure to ureaplasmas, but this was not significant (Fig. 5F).

Structural protein expression in the lung. The elastin foci on alveolar septa were counted on the lung sections (Fig. 6, A–D). In the ureaplasma 14-day fetal lungs, decreased numbers of
elastin foci were observed. The elastin was localized more diffusely compared with lungs after no exposure or shorter ureaplasma exposure times. A small increase of elastin foci was seen in ureaplasma 3-day fetal lungs (Fig. 6E). The lungs of ureaplasma 3-day animals inflated less well, leading to a higher density of the lung and the apparent increase in elastin foci.

The intensity of α-SMA staining was measured for arteries and bronchioli in the lung sections (Fig. 7, A–D). α-SMA staining was increased in arteries of ureaplasma 14-day fetuses (Fig. 7E). In bronchioli, α-SMA expression was increased in ureaplasma 3-day fetuses and remained high in ureaplasma 7- and 14-day fetuses (Fig. 7F).

**DISCUSSION**

The effect of short-term intrauterine ureaplasma exposure on lung development has previously only been studied after postnatal ventilation treatment in preterm baboons (51). In this study, we show that short-term exposure of fetal lambs to *U. parvum* serovar 3 in utero induced an inflammatory response and structural changes in the lung at 124 days GA. Neutrophils were recruited into the alveolar compartment of the lungs within 3 days of intra-amniotic ureaplasma injection. After 7 days of exposure to ureaplasmas, the neutrophils in the lung decreased, and after 14 days, the MHC class II-positive cells increased, although macrophage levels were not elevated. Simultaneously, T lymphocytes were increased in the PMLN, which was reflected in a tendency for increased weight of the PMLN. This pulmonary inflammation was not associated with an increase in inflammatory cell counts in cord blood, which is consistent with previous reports of intra-amniotic ureaplasma exposure in other animal models (35–37, 40). Long-term exposure to *U. parvum* serovar 3 or 6 (21–70 days of exposure, surgical delivery at 125 days GA) did not elicit an increase in inflammatory cells in cord blood of fetal lambs despite a marked increase in the inflammatory cell count in BALF (35–37). In fetal rhesus macaques inoculated with *U. parvum* serovar 1 at 4–9 days before delivery at 145 days GA (term = 167 days GA), leukocytes were strongly increased in the AF and the fetal lungs (40).

Interestingly, macrophages were not involved in the pulmonary inflammatory response to ureaplasmas in our study. The lack of a macrophage response to this pulmonary inflammation is quite different from the inflammatory response that is induced by intra-amniotic LPS in fetal lambs (26, 27). With LPS as the agonist, immature monocytes express PU.1 and mature...
to macrophages within days of exposure to LPS. The differences between an LPS- and a ureaplasma-induced inflammatory response are further illustrated by the observation that the cytokines IL-1β and IL-6 and surfactant proteins A, B, and C were not significantly increased and that there was an absence of lymphocytes in the lungs after exposure to ureaplasma, whereas these factors did increase after LPS exposure (1, 24, 29). This is supported by a study performed by Menon and colleagues (34), who reported that in cultured human fetal membranes, ureaplasmas caused a much milder cytokine response than LPS. In this model, ureaplasmas only triggered an increase in levels of TNFα and IL-10. Fetal sheep neither make much TNFα nor respond to TNFα (15, 21). Perhaps the different responses of the innate immune system result from how and when the immune system makes first contact with the microbe (11). LPS is a substance found on the cell wall of gram-negative bacteria and is recognized by a different array of pattern recognition receptors than bacteria like ureaplasma, which do not have cell walls. LPS triggers the immune response by binding to the CD14 receptor on antigen-presenting cells, leading to activation of Toll-like receptor (TLR) 4 and the myeloid differentiation factor-88 (MyD88)-dependent and -independent pathways. This subsequently leads to early and late NF-κB activation, inducing proinflammatory gene expression repetitively (11). Conversely, ureaplasmas activate NF-κB via TLR 1, 2, and 6 signaling with lipoproteins, predominantly the multiple-banded antigen, on its cell membrane (46). It also binds to mannose-binding lectin through terminal mannose residues (3), activating the lectin pathway of complement activation. These differences in the way the immune response is activated may very well lead to the different inflammatory phenotypes in the fetus.

In the developing fetal lung, alveolar septation is orchestrated by a number of structural proteins, among which elastin and α-SMA play important roles (4, 9, 25, 30, 56). Clustering of elastin and α-SMA at the tips of alveolar septa identifies the sites of budding for new alveoli (9, 56). If immature lungs are exposed to mechanical ventilation or chorioamnionitis at this point during development, focal elastin expression is diminished, and a more diffuse elastin expression develops along the alveolar cell wall (4, 25). As a result, a simplification of lung structure is observed, with fewer but larger alveoli. This phenomenon has been reported in mechanically ventilated infants and in patients with BPD (4–7, 9, 48, 49). Infants with BPD also have increased numbers of α-SMA-positive cells, or myofibroblasts, in their lungs, in relation to the severity of the

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**Fig. 7.** Expression of α-smooth muscle actin (α-SMA) in the lungs. Expression of α-SMA in the arteries of controls (A) and after exposure to UP for 14 days (B) and in the bronchioli of controls (C) and after 3 days of UP exposure (D) before delivery at 124 days GA. E: the intensity of α-SMA staining in the pulmonary vascular wall was increased 14 days after UP exposure. F: the intensity of α-SMA staining in bronchioli was increased 3 days after UP exposure. *P < 0.05 vs. controls using a 1-way ANOVA with Dunnett post hoc test.
disease (50). Furthermore, ureaplasma respiratory tract colonization in premature infants was associated with an increased incidence of BPD (13, 44, 52). Therefore, we tested whether changes in elastin organization and α-SMA expression occurred in premature lungs that were exposed to ureaplasmas. A decrease in foci of concentrated elastin on alveolar septa was noted 14 days after exposure to ureaplasmas, along with a more diffuse, dysmorphic expression of elastin elsewhere in the alveolar walls. α-SMA expression increased in pulmonary arteries and bronchioli after exposure to ureaplasmas. This decrease in elastin foci and elevated α-SMA expression in arteries and bronchioli may disrupt the further development of the lung. These findings are consistent with observations in preterm, ventilated, ureaplasma-infected baboons by Viscardi and colleagues (51). In contrast, it was recently reported that long-term exposure to U. parvum serovar 3 or 6 (70 days of exposure, delivery at 125 days GA) did lead to a functional maturation in the lungs but without causing sustained effects on air space and vascular development in fetal lambs (42). The effects of resuscitation, mechanical ventilation, and oxygen therapy need to be further studied to determine the effects on the long-term pulmonary outcome (17).

This study raises the question as to whether other sites of first contact, like the developing fetal skin and gastrointestinal tract, respond to ureaplasmas similarly. The developing gut in sheep is affected by exposure to LPS (55), however, it is unclear whether this is also true for ureaplasma exposure.

Our results support the hypothesis that ureaplasmas induce an acute inflammatory response in the fetal lung as a site in direct contact with the contaminated AF. Our findings suggest that a short-term exposure to ureaplasmas is sufficient to induce structural changes in elastin deposition antenatally, which may affect further alveolarization of the lung. This would explain why postnatal administration of antibiotics to counteract the ureaplasma infection has not been found to improve lung function (19, 32, 33).

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DISCLOSURES

No conflicts of interest, financial or otherwise, are declared by the author(s).

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