Intraamniotic Interleukin-1 Accelerates Surfactant Protein Synthesis in Fetal Rabbits and Improves Lung Stability after Premature Birth

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Abstract

Intraamniotic infection is associated with increased IL-1 activity in amniotic fluid, increased incidence of preterm labor, and with decreased incidence of respiratory distress syndrome in infants born prematurely. We hypothesized that an elevated IL-1 in amniotic fluid promotes fetal lung maturation. On day 23 or 25 of gestation (term 31 d), either IL-1α (150 or 1,500 ng per fetus) or its antagonist IL-1 receptor antagonist (IL-1ra, 20 μg) was injected to the amniotic fluid sacs in one uterine horn, whereas the contralateral amniotic sacs were injected with vehicle. Within 40 h, IL-1α caused a dose-dependent increase in surfactant protein-A (SP-A) and SP-B mRNAs (maximally, fivefold), without affecting lung growth or increasing inflammatory cells in the lung. Both genders, and upper and lower lung lobes were similarly affected. IL-1ra did not modify SP-A, -B, or -C mRNA. IL-1 increased the intensity of staining of alveolar type II cells for SP-B, and the concentrations of SP-B, -A, and disaturated phosphatidylcholine in bronchoalveolar lavage. The dynamic lung compliance and the postventilatory expansion of lungs were increased two- to fourfold after IL-1α treatment. In fetal lung explants, IL-1α increased the expression of SP-A mRNA. IL-1 in amniotic fluid in preterm labor may promote lung maturation and thus be part of a host-defense mechanism that prepares the fetus for extrauterine life. (J. Clin. Invest. 1997; 99:2992–2999.)

Key words: pulmonary surfactant • cytokine • respiratory distress syndrome • inflammation • prematurity

Introduction

Pulmonary surfactant is a complex of lipids and proteins synthesized and secreted by the type II alveolar cells into the alveolar epithelial lining where surfactant components act to reduce surface tension at the air–liquid interface. The surfactant lipids, predominantly dipalmitoyl phosphatidylcholine, are critical for generation of low surface tension. Some of the surfactant proteins that comprise up to 10% of the total mass of surfactant are also essential for surfactant function. Four surfactant proteins (SP) have been characterized to date: SP-A, -B, -C, and -D. SP-A is quantitatively the major surfactant apoprotein. The hydrophobic proteins, SP-B and -C, enhance the adsorption, spreading, and stability of the interfacial film (1, 2). SP-A binds to surfactant phospholipids and, together with SP-B, enhances the rate of adsorption of dipalmityl phosphatidylcholine to the air–liquid interface (1, 3). SP-A and -D are mannose binding proteins of the collectin family that are involved in host defense of inflammatory cells (4).

IL-1 denotes two polypeptides, IL-1α and IL-1β, that function as regulators in both local and systemic inflammatory reactions (5). IL-1α and IL-1β bind to the same cell surface receptors and have similar biological activities. IL-1 participates in host defense mechanisms, particularly in immunologic and hematologic responses, and upregulates the production of vasoactive mediators (PGF, nitric oxide, and others) (6). IL-1 can enhance its own production and that of a wide variety of other cytokines that in multiple ways modulate immune and inflammatory processes (5). Overproduction of IL-1 is likely to be detrimental and leads to depletion of normal physiological functions (6). However, IL-1 at low concentrations increases natural resistance to infection in animals (7–9). Activated macrophage monocyte is the major source of IL-1; however, B lymphocytes, endothelial, epithelial, mesangial, and smooth muscle cells and fibroblasts also synthesize IL-1 (5). IL-1 has been shown to be produced by decidual cells (10) and the amniochorion (11).

IL-1 receptor antagonist (IL-1ra) is a cytokine that is structurally related to IL-1α and IL-1β, but functions as an IL-1 inhibitor by competing with IL-1 for occupancy of the IL-1 receptor without inducing signal transduction (12). IL-1ra has been shown to block various IL-1–induced responses on many cells (12). In animal models of various inflammatory diseases, administration of IL-1ra reduces disease severity (5). IL-1ra may be a beneficial treatment for patients with septic shock, although a large clinical trial of IL-1ra treatment in sepsis did not show increased survival in the IL-1ra–treated group (13). Total blockade of IL-1 activity by IL-1ra in infection may in certain instances be harmful (14, 15). Monocytes and macrophages, including alveolar macrophages (16), produce and release IL-1ra that is also produced by gestational tissues, such as amnion and decidual cells (17).

Human amniotic fluid during the third trimester contains a high IL-1ra concentration (18). Amniotic fluid IL-1α and IL-1β concentrations, on the other hand, are usually low in normal gestation, but are elevated in patients with preterm labor oc-


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1. Abbreviations used in this paper: BAL, bronchoalveolar lavage; DPC, disaturated phosphatidylcholine; IL-1ra, IL-1 receptor antagonist; SP, surfactant protein.
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The animals were then placed in a series of plastic, 37°C temperature-controlled ventilator plethysmographs (24). The rabbits were ventilated for 30 min with 100% O₂ at a rate of 40 breaths/min, with an inspiratory time of 0.5 s and a positive end expiratory pressure of 1 cm H₂O. The peak inspiratory pressures were individually regulated to adjust the tidal volume to 8–10 ml/kg, as measured with a pneumotachometer and a differential pressure transducer (DP103; Validyne Engineering Corp., Northridge, CA). The maximum inspiratory pressure used was 40 cm H₂O. The compliance was calculated by dividing the tidal volume with the difference between the peak inspiratory pressure and the end expiratory pressure. After 30 min ventilation, the animals were killed with intracerebral pentobarbital, and the endotracheal tube was briefly plugged. Thereafter, the tracheal cannula of each animal was connected to equipment for parallel static pressure–volume recordings. Lungs were inflated through an endotracheal tube in 5 cm H₂O pressure increments to 35 cm H₂O, and then lowered stepwise to 0 cm H₂O with 30 s of stress relaxation at each 5 cm H₂O level. After the quasi-static pressure–volume measurement, the chest cage was opened, the diaphragm was inspected for evidence of pneumothoraces, and the lungs were inflated through an endotracheal tube to 30 cm H₂O for 30 s. The airway pressure was decreased to 10 cm H₂O, and the lungs were immersed in 4% formalin fixative. The aeration of the lungs was viewed on the sagittal sections of the lung, stained with hematoxylin and eosin. The volume density of the alveolar (empty) compartment was determined from the histologic sections by point counting, using total lung parenchyma as reference volume (25).

When indicated, the fetal animals were killed at birth, the chest cage was opened, intracardiac blood was recovered with a heparinized syringe, the trachea was cannulated, and BAL was performed by instilling the airways with 20–30 ml/kg 0.9% NaCl and thereafter recovering any fluid by gentle suction. This procedure was repeated four times. The total volume of combined BAL return was recorded.

RNA extraction and Northern blot analysis of surfactant proteins. Total RNA from lung tissue was isolated using the single-step acidic guanidium isothiocyanate method (26). RNA was quantitated by determining the absorbance at 260 nm, size separated in a 1.2% agarose, 5% formaldehyde gel, transferred to a nylon hybridization membrane (Genescreen; Dupont-NEN, Boston, MA), and cross-linked onto the membrane by ultraviolet light. Membranes were hybridized with random primer 32P-labeled probes using a 1.9-kb rabbit SP-A cDNA (a kind gift from Drs. C.R. Mendelson, Univ. Texas, Dallas, TX, and J.M. Snyder, Univ. Iowa Coll. Med., Iowa City, IA), a 1.7-kb rabbit SP-B cDNA (a gift from Dr. F. Possmayer, Univ. Western Ontario, London, Ontario, Canada), and a 0.5-kb rabbit SP-C cDNA (a kind gift from Dr. J.M. Snyder, and autoradiographed. Prehybridization and hybridization were performed in a hybridization incubator (100; Robbins Scientific Corp., Sunnyvale, CA) at 42°C; hybridization solutions contained 50% formamide, 5× Denhardt’s solution, 10% dextran sulfate, 1% SDS, and 0.1 mg/ml salmon sperm DNA in 0.75 M NaCl, 5 mM EDTA, and 50 mM sodium phosphate, pH 7.4. Posthybridization washes were performed once in 2× SSC, 0.1% SDS at ambient temperature for 5 min, twice in 0.2× SSC, 0.1% SDS, at 55°C for 25 min each, and final wash three times in 0.2× SSC, 0.1% SDS, at 55°C for 25 min each. To compensate for gel loading artifacts, all membranes were probed with 32P-labeled rabbit cytochrome oxidase subunit II (CO II) cDNA (a kind gift from Dr. S. Horowitz, Univ. Rochester, Rochester, NY). The bands were analyzed by video imaging and densitometry using a Macintosh computer and the public domain application National Institutes of Health Image software. Densitometric measurements were normalized to CO II mRNA before making comparisons between lanes in the autoradiograph.

Immunohistochemistry of SP-B. The tissues were fixed by immersion in 4% neutral buffered formaldehyde and embedded in paraffin. 5-μm sections were fixed to glass slides with glue, deparaffinized, and rehydrated. Endogenous peroxidase was blocked with 0.75% H₂O₂ for 40 min in room temperature and with 0.05% trypsin in PBS for 20 min at 37°C to unmask antigenic determinants. Non-specific immunoglobulin binding sites were blocked with 5% horse serum. Sections were then washed and incubated for 2 h at 37°C with the antibody against SP-B (dilution 1:2,000 in PBS containing 0.1% BSA). Negative controls consisted of nonimmune mouse IgG. Washed sections were then incubated with biotinylated horse anti–mouse antibody (1:400) for 30 min, washed, and incubated for 15 min with 1:400...
Table I. Body and Lung Weights and Contents of Protein, DNA, Phospholipid, and Disaturated Phosphatidylcholine in the Right Lungs of 25-d-old Fetal Rabbits Treated Intraamniotically with IL-1α or Placebo on Day 23.3 of Gestation*

<table>
<thead>
<tr>
<th></th>
<th>IL-1α (1,500 ng/fetus)</th>
<th>IL-1α (150 ng/fetus)</th>
<th>IL-1α (20 μg/fetus)</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Drug</td>
<td>Placebo</td>
<td>Drug</td>
</tr>
<tr>
<td>Fetal body weight (g)</td>
<td>16</td>
<td>14</td>
<td>17</td>
</tr>
<tr>
<td>Right lung:</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Weight (g)</td>
<td>0.28±0.04</td>
<td>0.24±0.03</td>
<td>0.23±0.02</td>
</tr>
<tr>
<td>Total protein (mg)</td>
<td>8.8±1.5</td>
<td>8.8±1.1</td>
<td>8.9±0.7</td>
</tr>
<tr>
<td>DNA (mg)</td>
<td>1.02±0.21</td>
<td>0.90±0.19</td>
<td>1.04±0.08</td>
</tr>
<tr>
<td>Total phospholipid (μmol)</td>
<td>2.16±0.29</td>
<td>1.75±0.34</td>
<td>2.01±0.25</td>
</tr>
<tr>
<td>DPC (μmol)</td>
<td>0.33±0.02</td>
<td>0.24±0.03</td>
<td>0.32±0.32</td>
</tr>
</tbody>
</table>

*The amniotic fluids of one uterine horn in each litter were injected with the drug, and the fetuses in the contralateral horn were treated with placebo.

Five litters were studied in each treatment group. Values are the means per fetus for each uterine horn±SEM. The number of fetuses in each group is indicated. *P ≤ 0.05 compared with placebo treatment.
Results

Fetal weight, lung weight, lung phospholipid, and DNA content. There were no differences in the weights of the fetuses between the treatment groups of fetal rabbits (gestational age 25 d) treated intraamniotically with IL-1α or vehicle on d 23.3 of gestation. Lung weight, protein, and DNA content were likewise similar in the different groups (Table I).

The total phospholipid content was higher in the lungs of fetuses treated with a high dose of IL-1α (1,500 ng/fetus) than in those of the controls (Table I). There was also a tendency for the IL-1α–treated animals to have a higher DPC content in their lungs (P = 0.05). In the group treated with the lower IL-1α dose (150 ng/fetus), the phospholipid content also tended to be higher, but this difference was not significant. The group treated with IL-1ra did not differ from the corresponding control group with respect to lung phospholipid or DPC content (Table I).

SP-A, -B, and -C mRNA in fetal rabbit lungs on day 25. Fig. 1 shows the mRNA expression of SP-A, -B, and -C in the lungs of IL-1α–treated and the control fetuses. There were no differences in the expression of any of the three surfactant proteins between the IL-1ra–treated and the control fetuses. IL-1α stimulated in a dose-dependent fashion the expression of SP-A and -B mRNA. For each cytokine dose, the results of six litters of fetuses are shown. IL-1α stimulated in a dose-dependent fashion the expression of SP-A and -B mRNA. There was no difference in surfactant protein expression between the upper and lower lobes and between female and male fetuses (data not shown).

Fig. 3 shows the surfactant protein expression in fetuses treated with IL-1ra. The data is obtained from five litters of fetuses (data not shown).

Immunohistochemistry for SP-B, and surfactant components in BAL. Immunostaining for SP-B was more intense in IL-1α–treated than in control lungs (Fig. 4). In both groups, several fields from four lungs were evaluated. The percentage of alveolar epithelial cells stained for SP-B from IL-1α–treated animals was 56±13%; this was not significantly higher than vehicle-treated animals (42±9%). IL-1 treatment did not increase the number of inflammatory cells in the lung.

IL-1α–treated fetuses had a higher concentration of SP-B in BAL return than the placebo-treated ones. DPC concentrations in BAL return were also increased in fetuses treated with IL-1α (Table II). Similar to SP-B, the SP-A staining of the SDS gels was more prominent after IL-1α than after vehicle, with the glycosylated SP-A (36 kD) being the main component in each case (data not shown).

Lung compliance and the expansion patterns of the lungs. Fetuses were treated with IL-1α (1,500 ng/fetus) or the corresponding vehicle on day 25 of gestation. On day 27 of gestation, the fetuses were ventilated in total body plethysmographs as described in Methods. The dynamic lung compliance measured after 2 min of ventilation of IL-1α–treated fetuses was significantly greater than that of control fetuses (Fig. 5).

Figure 2. SP-A, -B, and -C mRNA expression in lungs of 25-d-old fetuses treated with intraamniotic IL-1α. Results from six litters are shown for each cytokine dose.

Figure 3. SP-A, -B, and -C mRNA expression in lungs of 25-d-old fetuses treated with intraamniotic IL-1α (20 μg/fetus). Results from five litters of fetuses are shown.
mRNA, whereas IL-1α had no effect on SP-B mRNA in vitro (data not shown).

Discussion

The present study shows for the first time that intraamniotic administration of a cytokine modulates lung maturation, without affecting fetal lung growth. IL-1α accelerated the expression of SP-A and -B, but did not affect SP-C mRNA. IL-1α also stimulated the expression of SP-A in fetal rabbit lung explants in culture, whereas under these conditions SP-B was unaffected. On the other hand, IL-1ra, which is present in high concentration in amniotic fluid (18), did not modify the expression of SP-A, -B, or -C mRNA when given in excess intraamniotically to fetal rabbits or added to fetal lung cultures (34). This suggests that endogenous IL-1 activity does not play a role in the expression of surfactant proteins in fetal lung during normal preterm gestation.

Various proinflammatory cytokines accumulate in amniotic fluid in labor associated with intrauterine infection (19, 35). In this condition, the concentration of IL-1 (IL-1α + IL-1β) is within the range required for acceleration of surfactant protein synthesis in fetal lung explants, and attainable with intraamniotic injections of 150–1,500 ng of IL-1α to fetal rabbits (19). Proinflammatory cytokines participate in the onset and progression of labor. In the present study, no cases of preterm birth occurred during the 40-h period after IL-1α treatment. An increased incidence of preterm birth, induced by proinflammatory cytokines (combination of IL-1α and TNF-α) occurs first 3 d after intraamniotic cytokine injections in rabbits (36).

Surfactant synthesis is regulated by several hormones, most notably glucocorticoid (37). Depending on the concentration, corticosteroids stimulate or inhibit the expression of SP-A in human fetal lung explants (38). Administration of dexamethasone to pregnant rats upregulates the expression of SP-A and to a lesser degree that of SP-B and -C (39). Administration of glucocorticoid to the doe or the fetus decreases fetal lung growth (37).

In addition to hormones, some cytokines have been proposed to have a role in the differentiation of alveolar type II cells. EGF, TGF-α, IL-1, and IFN-γ stimulate the production of various surfactant components. EGF enhances the rate of choline incorporation into disaturated phosphatidylcholine in explants of fetal rat lung (40) and stimulates phosphatidylcholine synthesis in alveolar type II cells isolated from fetal rabbit lung (41). In explants of human fetal lung obtained between 15 and 24 wk of gestation, EGF stimulates the synthesis of SP-A and the expression of SP-A mRNA (42). Intraamniotic and simultaneous intraperitoneal injection of EGF increases SP-A concentration as well as the lecithin/sphingomyelin ratio in the amniotic fluid of fetal rhesus monkeys (43). IFN-γ increases SP-A expression in human fetal lung explants (44) and in adenocarcinoma cells (45). On the other hand, cytokines such as TNF-α and -β downregulate the synthesis and expression of surfactant proteins and lipids. TNF-α decreases expression of

**Table II. The Concentrations of the Phospholipids and Surfactant Protein B in BAL from 27-d-old Fetuses Treated Intraamniotically with IL-1α or Placebo on Day 25.3 of Gestation**

<table>
<thead>
<tr>
<th></th>
<th>IL-1α (1500 ng/fetus)</th>
<th>IL-1α (150 ng/fetus)</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Drug</td>
<td>Placebo</td>
</tr>
<tr>
<td>n</td>
<td>9</td>
<td>8</td>
</tr>
<tr>
<td>Total phospholipids (μM)</td>
<td>62.3±4.3</td>
<td>48.0±5.2</td>
</tr>
<tr>
<td>Phosphatidylcholine (μM)</td>
<td>49.3±6.8</td>
<td>36.4±5.4</td>
</tr>
<tr>
<td>Disaturated phosphatidylcholine (μM)</td>
<td>24.3±3.8</td>
<td>14.1±1.8</td>
</tr>
<tr>
<td>Surfactant protein B (ng/ml)</td>
<td>188±25*</td>
<td>90±17</td>
</tr>
</tbody>
</table>

Values are mean±SEM. *P < 0.05 compared with placebo treatment.
Intraamniotic Interleukin-1 Accelerates Lung Maturation

SP-A and -B in human pulmonary adenocarcinoma cells (45) and in human lung explants (46). TNF-α also inhibits phosphatidylcholine synthesis in human type II alveolar cells isolated from adult human lung tissue (47). Likewise, TGF-β inhibits the expression of SP-A mRNA and production of SP-A protein in explants of human fetal lung (42) as well as in pulmonary adenocarcinoma cells in culture (48).

Intraamniotic IL-1 may act on the expression of surfactant proteins by several mechanisms. The cytokine may reach the alveoli either directly by fetal breathing movements or by being absorbed into the fetal circulation from the gastrointestinal tract or from fetal membranes. It is possible that IL-1 has a direct stimulatory effect on the synthesis and secretion of surfactant components by type II cells. IL-1 may also act on other fetal cells, such as the alveolar macrophage or the type II alveolar cell to stimulate the production of another agent such as PGE (6), known to stimulate SP-A expression (49). In addition, IL-1 in amniotic fluid may induce in amnion cells the production of mediators such as PGE2, which in turn may promote surfactant protein expression in type II cells.

It can be envisaged that IL-1 increases the production of ACTH-releasing hormone and glucocorticoids by the fetus (50). Activation of the hypothalamic–pituitary–adrenal axis by IL-1 has been shown to occur in newborn animals (51). Stimulation of corticosteroid production would therefore possibly contribute to enhanced expression of surfactant components (37–39). In young animals, corticosterone production in response to IL-1 shows sexual dimorphism, with female animals releasing more corticosterone in response to the cytokine than do males (52). This is of interest in view of the more advanced maturation of the surfactant system occurring in mammalian females and of an apparently higher responsiveness of the immature female lung to factors stimulating the synthesis of DPC (53). On the other hand, human female fetuses have higher IL-1rα concentrations in the amniotic fluid than do males (18). It remains to be studied whether maternal chorioamnionitis affects the gender difference in lung maturation. In the present study, injection of IL-1 into the amniotic fluid did not lead to elevated cortisol levels in fetal plasma (IL-1α: 4.14 ± 0.22 ng/ml vs. vehicle 3.61 ± 1.17, n = 5). There was no gender difference in the expression of surfactant proteins among the IL-1α- or placebo-treated fetuses.

Activation of the glucocorticoid axis is not likely to be the sole mechanism of IL-1-induced functional maturation of the lung, since IL-1 increased SP-A expression also in lung explants in vitro. IL-1 is known to affect either the stability (54) or the transcription rate (55) of specific genes. Activator protein-1 is one of the nuclear transcription factors that, as a result of IL-1-induced activation, binds to specific consensus sequences, enhancing the gene transcription rate (55). A highly conserved activator protein-1 consensus was recently identified upstream from the transcription initiation site of the SP-D gene (56). However, a similar sequence has thus far not been associated with the SP-A or -B gene. In spite of the clear cut

Figure 5. Dynamic lung compliance of IL-1α–treated (1,500 ng/fetus) and vehicle-treated 27-d-old fetal rabbits (4 litters, 25 animals) ventilated in total body plethysmographs. After 2 min of ventilation, IL-1α–treated animals had higher compliance than the controls (P < 0.05).

Figure 6. Expansion patterns of ventilated lungs from 27-d-old fetal rabbits: from an IL-1α–treated (1,500 ng) fetus (A) and from a vehicle-treated littermate (B).
stimulation of SP-B mRNA and SP-B content in immature lung in vivo, the failure of IL-1 to affect SP-B mRNA in vitro supports the possibility that this gene was upregulated by an extrapulmonary secondary mediator.

IL-1 concentrations are elevated in amniotic fluid in patients in premature labor with intraamniotic infection (19). Here we show that intraamniotic IL-1 increased SP-B and di-saturated phosphatidylcholine in the lung. These surfactant components are required for alveolar stability (1, 2). In addition, IL-1 increased SP-A that, besides promoting the surface activity (24, 27), together with SP-D enhances the micobidial activity of alveolar macrophages in vitro (4). Consistent with these findings, we demonstrated a striking improvement in dynamic compliance and homogeneous alveolar expansion of ventilated preterm animals after intraamniotic IL-1ox. In contrast, intramuscular injection of IL-1ox (1.5–1.500 ng) to rabbit fetus had no detectable effect on the expression of surfactant proteins (K. Bry et al., unpublished observations). By promoting lung maturation, accumulation of IL-1 in the amniotic fluid may be a part of a host-defense mechanism that prepares the fetus for preterm birth and extraterritorial life.

Acknowledgments

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References


