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Prenatal Exposure to Epidermal Growth Factor Attenuates Respiratory Distress Syndrome in Rhesus Infants[1]

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ABSTRACT. Treatment of nonhuman primate fetuses with epidermal growth factor (EGF) results in histologic and biochemical maturation of their lungs. To determine whether these effects improve lung function postnatally, we studied premature rhesus infants delivered at 78% of gestation after *in utero* treatment with EGF ($n = 5$) or placebo ($n = 5$). Indices of lung function during the 4 d of postnatal care included fractional concentration of inspired oxygen, peak inspiratory pressure, ventilator rate, mean airway pressure, arterial to alveolar oxygen tension ratio, and ventilation index. Statistically significant differences were noted in the time courses of these variables between EGF- and placebo-treated infants. The direction of the differences indicated that the EGF-treated infants had less severe lung disease. Surfactant apoprotein A concentration and lecithin to sphingomyelin ratio were both significantly higher in the amniotic fluid of the EGF-treated group, indicating advanced biochemical maturation in this group of animals. Whereas birth weight was not affected by EGF exposure, adrenal and gut weights, standardized for body weight, were increased significantly. Histologic studies showed advanced cellular maturation with increased parenchymal airspace and decreased parenchymal tissue space in the EGF-treated group compared with the control group. We conclude that prenatal exposure to EGF stimulates biochemical and histologic maturation of the lung and markedly attenuates the clinical severity of respiratory disease in this model of simian respiratory distress syndrome. (*Pediatr Res* 35: 30–36, 1994)

Abbreviations

a/A O_2, arterial to alveolar oxygen tension ratio
BPD, bronchopulmonary dysplasia
EGF, epidermal growth factor
FIO_2, fractional concentration of inspired oxygen
GD, gestational day
MAP, mean airway pressure
$PaCO_2$, arterial blood carbon dioxide tension
PaO_2, arterial blood oxygen tension
PIP, peak inspiratory pressure
Rate, frequency of ventilator cycling
RDS, respiratory distress syndrome
VI, ventilation index

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EGF, a naturally occurring polypeptide, stimulates development of pulmonary epithelial cells as demonstrated by histologic maturation of alveolar type II cells in tissue culture and *in vivo* (1–5). EGF induces increased synthesis and secretion of surfactant and surfactant-associated proteins in these cells. However, there is only limited information as to whether histologic and biochemical maturation induced by EGF confers functional maturation. Fetal administration of EGF to rabbits and lambs increases distensibility of their lungs (6, 7). Preliminary observations suggest that respiratory distress is less severe in the preterm lambs treated *in utero* with EGF (7). However, there are no objective data regarding the effects of prenatal exposure to EGF on the time course of respiratory distress in any animal model with surfactant deficiency. Nonhuman primates delivered at 75–80% of term, including rhesus macaques, have been shown to have surfactant deficiency and associated respiratory distress similar to that seen in premature human infants with RDS (8–12). Therefore, we elected to study whether prenatal exposure to human recombinant EGF produces functional maturation of the lung sufficient to alter the course of RDS in prematurely delivered rhesus monkeys.

MATERIALS AND METHODS

All procedures used within this study conformed to the requirements of the Animal Welfare Act. The California Regional Primate Research Center is fully accredited by the Association for Accreditation of Laboratory Animal Care and all study protocols were approved before implementation by the Institutional Animal Use and Care Committee at the University of California at Davis. Activities related to animal care and surgery/necropsy were performed according to standard Primate Center operating procedures.

Gestational age and EGF administration. Normally cycling, female *Macaca mulatta* were bred midcycle with proven fertile males. Pregnancy was confirmed by assay of monkey chorionic gonadotropin or ultrasound on GD 20 ± 2 (13, 14). GD 0 was assigned as the last day of exposure to the male. A total of 11 gravid animals whose fetuses had appropriate-for-gestational-age femur length and biparietal diameter at GD 121 were selected for the study (14). Dams received ketamine hydrochloride (10

mg/kg) during fetal injections to immobilize them. Fetal monkeys received recombinant human EGF (15) (provided by Chiron Corporation, Emeryville, CA) or saline placebo on GD 121, 123, 125, and 127. Because the route of action of EGF on fetal lung development was not known, EGF (40 μg in 1.0 mL of saline) was injected into the amniotic fluid and also into the fetal peritoneal cavity under ultrasound guidance as previously described (16). Biochemical and histologic maturation of airway epithelial cells was previously demonstrated using this treatment regimen in fetuses of this gestational age (4). The average EGF dose was 533 μg/kg by each route over the 7-d period as calculated for an approximate average fetal body weight of 300 g. Control fetuses underwent an identical protocol with injections of saline placebo. The treatment status of each animal was assigned by one investigator (L.C.R.), and the personnel providing the neonatal intensive care remained blinded to the assignment until the infant was euthanized.

Neonatal intensive care. Rhesus infants were delivered on GD 128 (78% of gestation) by cesarean section with the dam under general anesthesia. After withdrawing a sample of the amniotic fluid, the fetal head was delivered, the trachea was cannulated with a 2-mm diameter endotracheal tube, and manual positive pressure ventilation was initiated with a self-inflating infant resuscitation device. The remainder of the fetal body was then delivered, the umbilical cord severed, and the infant transferred to a radiant warmer bed in the adjacent intensive care unit. The placenta and membranes were removed after delivery of the infant and their weights obtained.

Mechanical ventilation was continued with a human infant ventilator. The infant was thoroughly dried and ECG leads and oximetry sensors were applied to the extremities for monitoring. Umbilical arterial catheterization was performed with a 3.5 Fr polyvinyl chloride catheter to allow for blood pressure monitoring and continuous fluid and glucose infusion. Vital signs were recorded every 2 h and arterial blood gases were measured as needed but not less than every 6 h. The ventilator management strategy was to maintain the blood gases in the following ranges: Pa_{O_2} = 6.6–13.3 kPa (50–100 torr), Pa_{CO_2} = 4.7–7.3 kPa (35–55 torr), and pH = 7.25–7.45. Ventilator settings and FIO_2 were adjusted according to blood gas measurements. The end-expiratory pressure was maintained at 0.4 kPa (4 cm H_2O) and the inspiratory time was maintained at 0.35–0.40 s for the duration of the study. Blood chemistries were obtained for each blood gas sample using a whole-blood analyzer. Urine and blood were periodically tested for glucose. Body weight was obtained daily. Fluid infusion rates and electrolyte composition were adjusted in a manner similar to that described for human infants (17). Enteral feeding with 2 mL of human infant formula by gavage tube every 3 h began at 24 h of age and increased by 0.5 mL every 12 h as tolerated to a maximum of 5 mL per feeding. Replacement blood transfusion was performed with freshly drawn uncrossmatched adult rhesus blood each time a total of 3 mL of an infant's blood had been drawn for blood tests. Tracheal extubation was not attempted when low ventilator settings were achieved to avoid introducing additional confounding variables.

Lung function. Lung function was assessed by serial analysis of blood gases, FIO_2, and ventilator settings of PIP, positive end-expiratory pressure (PEEP), and Rate. Three derived variables, MAP, a/A O_2, and VI were determined according to the following equations:

$$MAP = (PIP \cdot I_t + PEEP \cdot E_t)/(I_t + E_t)$$

$$a/A\ O_2 = Pa_{O_2}/FIO_2(Patm - P_{H2O} - Pa_{CO_2}/RQ)$$

$$VI = PIP \cdot Rate \cdot Pa_{CO_2}/40$$

where I_t is inspiratory time, E_t is expiratory time, Patm is atmospheric pressure, and P_{H2O} is the vapor pressure of water. In addition, daily pressure-volume loops were recorded during muscular paralysis induced with parenteral vecuronium, 0.03 mg/kg, at fixed PIP of 15, 30, and 45 cm H_2O as described by Caeton *et al.* (18). Flow was measured with a size 00 Fleisch pneumotachograph interposed between the ventilator and the tracheal tube connected with a Statham PM197 differential pressure transducer (Gould Statham, Oxnard, CA). Airway pressure was measured by a Hewlett-Packard 1230 pressure transducer (Hewlett-Packard Co., Palo Alto, CA). These signals were fed to respective Hewlett-Packard 17403A amplifiers, and the analog signals were digitized by a computer so that the pressure-volume loops could be displayed and total respiratory compliance estimated (18).

At 90–96 h after delivery, the animals were euthanized with an overdose of pentobarbital and the lungs removed, trimmed of other mediastinal tissues, weighed, and prepared for biochemical and histologic studies. Total body weight, lung weight, and other organ weights were obtained at necropsy. The gut weight included the stomach, small intestine, cecum, and large intestine weights.

Histologic and morphometric measurements. After weighing, the lungs were separated at the carina. The right cranial lobe was cannulated and fixed by airway perfusion at 30 cm of fixative pressure using glutaraldehyde-paraformaldehyde in 0.2 M cacodylate buffer (adjusted to 330 mosmol and pH 7.4). The fixed volume of this lobe was measured by fluid displacement. Specific tissue areas, including airway levels and parenchyma, were selected by microdissection and processed for embedding in Araldite 502 (Electron Microscopy Sciences, Washington, PA). Volume densities of parenchymal air space and parenchymal tissue, alveolar size (mean linear intercept) and alveolar surface area (surface to volume ratio) were estimated by point and intercept counting of 1-μm sections using a light microscope and a graticule containing a Weibel 42-multipurpose test grid (×250 magnification) and by applying standard stereologic formulas. These measurements were performed only on lungs (four animals in each group) where sections of bronchioles were free of artifactual enfoldings as determined by light microscopy. A minimum of five parenchymal fields were selected from various regions of the right cranial lobe of each animal for evaluation.

Biochemical measurements. Amniotic fluid, obtained at the time of delivery, was centrifuged and frozen at −20°C for later analysis of surfactant-associated protein A and surfactant lipid profiles by previously reported biochemical techniques (19, 20).

Data analysis. The time courses for PIP, Rate, FIO_2, MAP, a/A O_2 ratio, and VI were compared by determining the areas under the respective curves for each animal between 1 and 90 h and comparing the group means using the *t* test (21). The mean values for these variables were also compared at 24, 48, 72, and 90 h using this test. The mean values for the categorical variables determined for the EGF-treated animals were compared with those for the saline-injected control animals using the *t* test for group means. No value was used in more than one comparison. A *p* value less than 0.05 for a two-tailed comparison was considered statistically significant. Values of variables to be compared are given as means ± SEM.

RESULTS

Dams were of similar mean body weights (7.2 ± 1.0 and 6.1 ± 1.5 kg for the control and EGF groups, respectively). One control infant died at 20 h of age from severe RDS complicated by a pneumothorax. Data collected from this animal were omitted from the comparisons to be presented. Two additional animals in the control group developed pulmonary air leaks that required chest tube drainage and both survived. Thus, five EGF-treated and five placebo-treated animals completed the entire study. There were three females and two males in each group.

The time courses for the blood gas measurements of Pa_{O_2}, Pa_{CO_2}, and pH for the EGF and control groups are presented in Figure 1. These data suggest that the control group animals were more difficult to maintain in the desired ranges of these variables

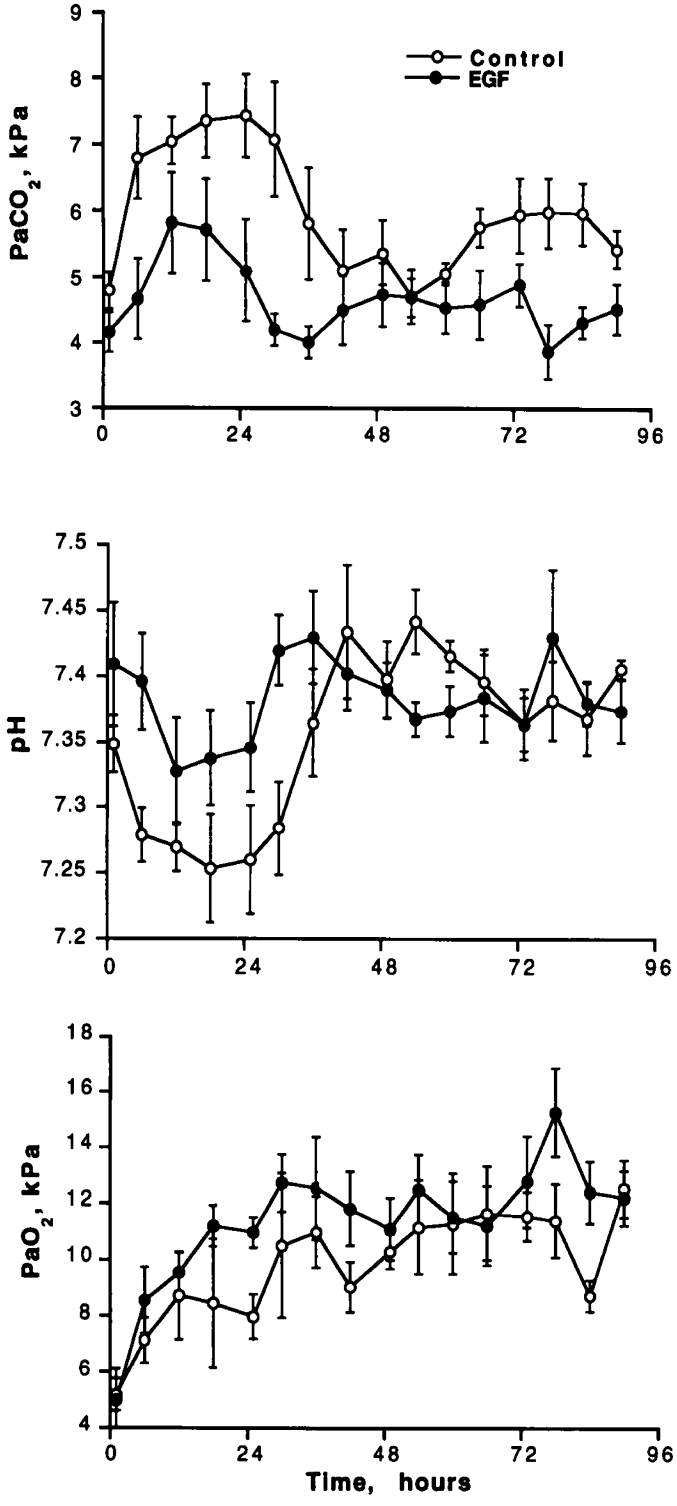

Fig. 1. The mean values (± SEM) for the blood gas measurements of Pa_{O_2}, Pa_{CO_2}, and pH over the 90-h duration of the study. The first time points are at 1 h after birth. The time courses of the EGF and control groups suggest that the control blood gas variables were easier to maintain within the desired ranges (see text).

because their mean Pa_{O_2} were slightly lower and their mean Pa_{CO_2} were slightly higher than those of the EGF-treated group. Furthermore, during the first 24 h, we were not uniformly successful in meeting our goals for CO_2 management for infants in the saline-treated control group. The blood gas variables were not compared statistically because we actively intervened to maintain them in predetermined ranges.

The time course and severity of the lung disease in the EGF and control groups are compared in Figures 2 and 3. Figure 2 compares the time courses of the supplemental oxygen requirement (FIO_2) and the ventilator settings of PIP and Rate. The

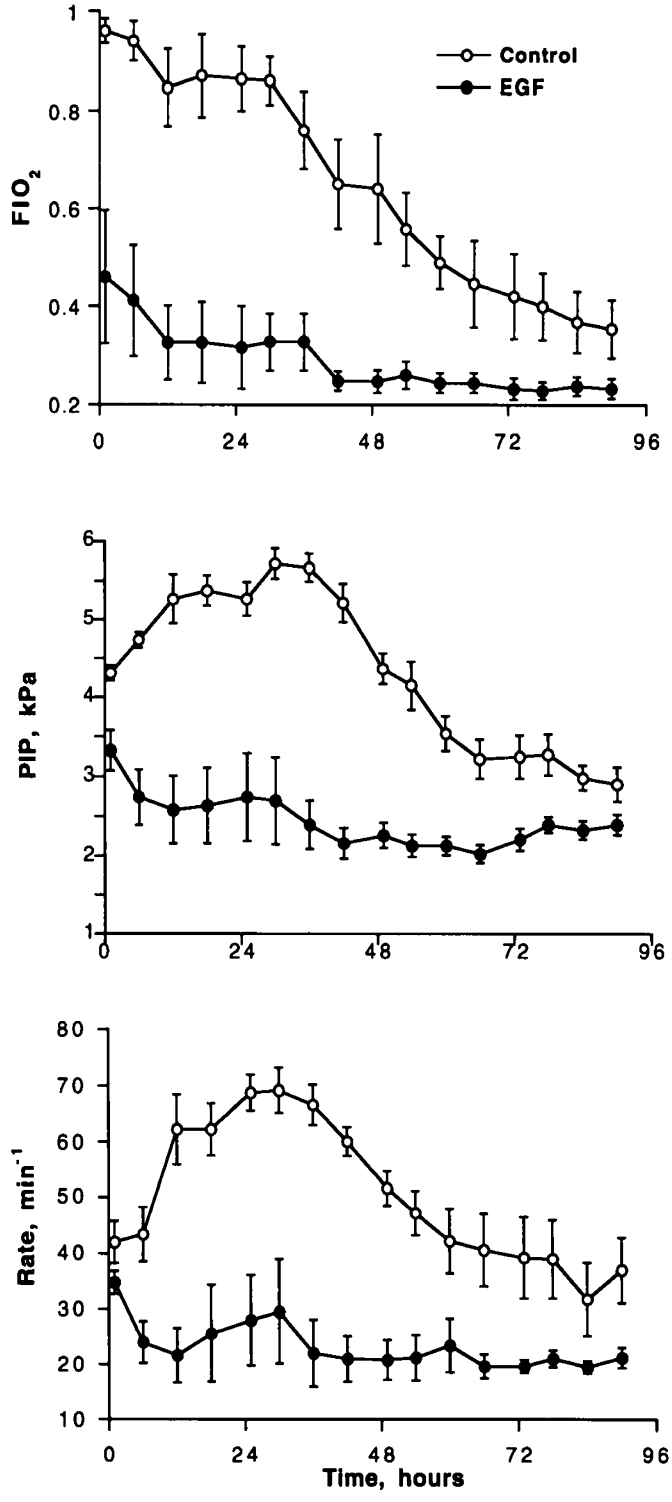

Fig. 2. The mean values (± SEM) for the ventilator variables of FIO_2, PIP, VI, and Rate over the 90-h duration of the study. The first time points are at 1 h after birth. The time courses of the EGF and control groups were statistically different with $p = 0.0002$ for the FIO_2 and PIP comparisons and $p = 0.0007$ for the Rate comparison. At 72 and 90 h, the FIO_2 and PIP measurements, respectively, ceased to be significantly different for the two groups, whereas the Rate means remained significantly different throughout the study.

time courses, analyzed by the areas under these curves, were significantly different for the two groups with p values of 0.0002, 0.0002, and 0.0007 for FIO_2, PIP, and Rate, respectively. Figure 3 compares the time courses of the derived variables of MAP, a/A O_2 ratio, and VI. These time courses for the EGF-treated animals were significantly different from those of the control group with p values of 0.0003, 0.002, and 0.0004 for MAP, a/A O_2 ratio, and VI, respectively. These data show that the EGF-treated animals required less ventilator support (PIP, Rate, MAP, and VI) and less supplemental oxygen (FIO_2 and a/A O_2 ratio) than the control animals. Thus, it was concluded that the EGF-treated animals had a less severe respiratory disorder according to these indices. In addition, the time course of the respiratory disease was shorter for the EGF-treated animals, as evidenced by their achieving low stable values for supplemental oxygen and ventilatory support by 42 h of age, whereas the control animals had not achieved this level of improvement at 90 h of age. The ventilator rate for the EGF-treated animals was significantly lower than that for the control group at 24, 48, 72, and 90 h. At 72 h, the FIO_2 and VI ceased to be significantly different for the two groups. At 90 h, the remaining variables of PIP, MAP, and a/A O_2 ratio ceased to be statistically different as the placebo-treated animals' lung disease spontaneously resolved. At the termination of the study, the mean FIO_2, PIP, and Rate for the EGF-treated group were 0.24 ± 0.04, 1.80 ± 0.21 kPa (18.0 ± 2.1 cm H_2O), and 21.2 ± 4.1 min^{-1}, respectively. The comparison values for the placebo group were 0.35 ± 0.13, 2.18 ± 0.36 kPa (21.8 ± 3.6 cm H_2O), and 37.0 ± 13.1 min^{-1}. In our clinical judgment, four of the five EGF-treated animals and two of the five placebo-treated animals would have tolerated extubation by 90 h of age and all 10 animals could have survived their respiratory disease.

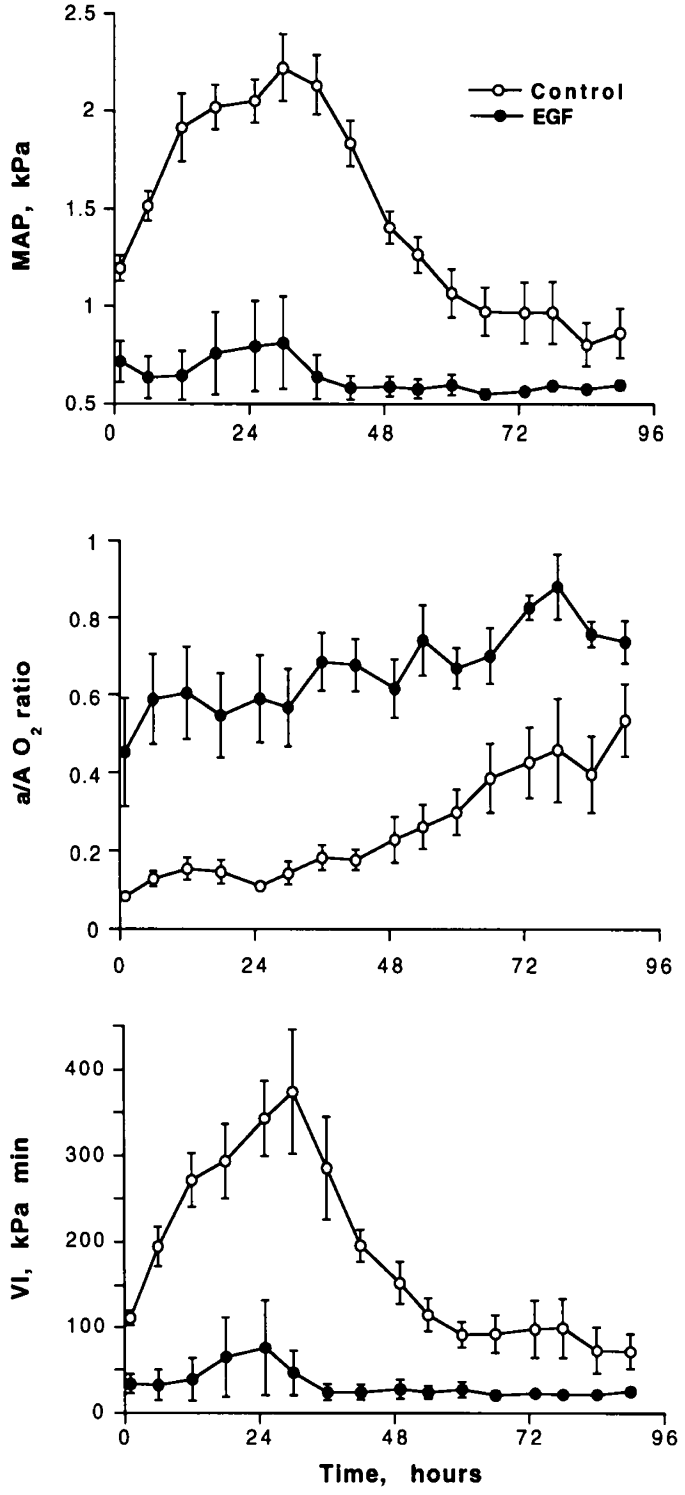

Fig. 3. The mean values (± SEM) for the derived variables of MAP, a/A O_2 ratio, and VI over the 90-h duration of the study. The first time points are at 1 h after birth. The time courses of the EGF and control groups were statistically different with $p = 0.0003$, 0.002, and 0.0004 for the MAP, a/A O_2, and VI comparisons, respectively. At 72 h, the VI ceased to be different for the two groups, and at 90 h, MAP and a/A O_2 ratio measurements ceased to be significantly different for the two groups.

Analysis of the amniotic fluid obtained at the time of delivery showed that the mean concentration of surfactant apoprotein A was significantly higher in the EGF-treated group than in the control group [18.9 ± 3.8 *versus* 3.8 ± 0.2 μg/mL ($p < 0.005$)] as was the mean lecithin to sphingomyelin ratio [2.8 ± 0.7 *versus* 1.2 ± 0.2 ($p < 0.05$)].

The total lung compliance values for the control and EGF-treated groups at 24 and 90 h of age are shown in Table 1. There were no significant differences within or between groups at these times or at the different inspiratory pressures.

The group mean body weights, organ weights, and organ weight to body weight ratios for selected organs are presented in Tables 2 and 3. No significant differences were detected between the two groups for birth weight, placenta to birth body weight ratio, or necropsy body weight. However, the adrenal to necropsy body weight and the gut weight to necropsy body weight were greater for the EGF-treated group ($p = 0.036$ and 0.002, respectively). The mean wet lung weight to necropsy body weight was slightly less for the EGF-treated group of animals, but the difference did not reach statistical significance ($p = 0.078$). Liver, kidney, and brain weights were unaffected by treatment with EGF. Parenthetically, there were no differences between the two groups for spleen, thyroid, and eye weights (data not shown).

Table 1. *Total respiratory compliance (C_t) at 24 and 90 h of age for control (n = 5) and EGF-treated (n = 5) rhesus infants**

Inspiratory pressure (kPa)	C_t control (mL/kPa/kg)		C_t EGF (mL/kPa/kg)	
	24 h	90 h	24 h	90 h
4.5	0.10 ± 0.03	0.07 ± 0.05	0.13 ± 0.04	0.08 ± 0.04
3.0	0.09 ± 0.02	0.07 ± 0.04	0.11 ± 0.04	0.08 ± 0.04
1.5	0.08 ± 0.02	0.06 ± 0.04	0.08 ± 0.04	0.07 ± 0.04

* Values are means ± SEM.

Table 2. *Body weights and placenta weights for control (n = 5) and EGF-treated (n = 5) rhesus infants**

	Control	EGF
Birth weight (g)	307 ± 9	315 ± 12
Placenta weight (g)	91 ± 5	90 ± 4
Necropsy weight (g)	298 ± 18	290 ± 11

* Values are means ± SEM.

Table 3. *Selected organ weight to necropsy body weight ratios for control (n = 5) and EGF-treated (n = 5) rhesus infants**

Organ	Control (g/kg)	EGF (g/kg)
Lung	26 ± 2.2	21 ± 1.8
Adrenal	0.710 ± 0.020	0.870 ± 0.004†
Gut	24 ± 2	34 ± 1†
Liver	37 ± 1	38 ± 1
Kidney	9.0 ± 0.4	9.0 ± 0.3
Brain	142 ± 12	148 ± 7

* Values are means ± SEM.
† $p < 0.05$, for EGF group *vs* control group.

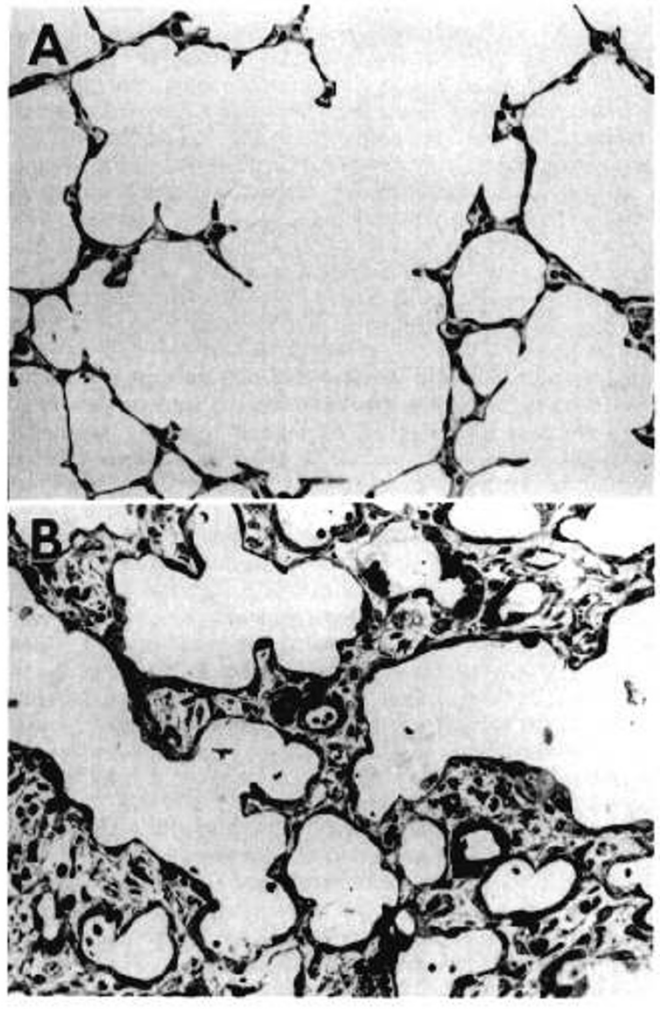

Fig. 4. Light microscopic comparison of lung parenchyma in rhesus monkey infants after 4 d of mechanical ventilation after *in utero* treatment with either EGF (*A*) or saline (*B*). Magnification = 210×. In EGF-treated infants, the alveolar air spaces were larger and more uniform in size than in the saline-treated control infants.

Histologic appearance of the parenchymal tissue differed between EGF and control animals (Fig. 4). The lungs of the control animals appeared to have smaller air spaces with larger interalveolar septa. The epithelium lining the air space side of the septa was thicker, more cuboidal, and appeared to be composed of more cells, and there was a wide range of septal thickness. In contrast, the EGF-treated animals had air spaces that appeared larger, more regular, and evenly sized throughout the lung specimens. The septa were thinner and the epithelium lining the surfaces was composed primarily of thin squamous cells intermixed with small numbers of cuboidal cells. Respiratory bronchioles of untreated animals contained exfoliated bronchiolar epithelium and other necrotic cells in the air spaces. The epithelium was not as regularly arranged or as tall as was observed in the treated animals. The percent of air space was greater in EGF-treated animals than in controls (Table 4), and the size of individual air spaces was larger in the EGF-treated animals compared with the controls. The percent of lung parenchyma occupied by tissue was less in EGF-treated animals (Table 4), although the surface area to volume ratio of tissue was approximately the same in both groups of animals.

DISCUSSION

Our results show that treatment of preterm rhesus monkey fetuses with EGF for 1 wk before delivery markedly attentuates the time course and severity of their postnatal respiratory distress. We confirmed that EGF advances structural and biochemical maturation of their lungs and conclude that this was sufficient to confer the functional advantage observed after their premature delivery.

Our study is the first to report the effects of EGF on the course of RDS in an animal model. However, several previous *in vivo* studies have shown that fetal administration of EGF may enhance pulmonary maturation. Catterton *et al.* (6) injected EGF, 200 μg/kg, into fetal rabbits at 24 d of gestation and demonstrated increased lung distensibility and stability on deflation after delivery 2 d later. They observed an increase in the alveolar type II cells lining the alveoli of EGF-treated pups, and these cells contained more lamellar bodies than the saline-treated control pups. Sundell *et al.* (7) infused EGF, 40–200 μg/kg/d, for 5 d into one of twin fetal lambs beginning at 123–125 d of gestation and the other twin received only a saline infusion. Maternal hypotension was induced on the 4th d of the EGF infusion and the lambs were delivered the next day and mechanically ventilated for up to 6 h. The control twins were reported to have more severe respiratory distress than the EGF-treated twins based on clinical findings. However, no blood gas or ventilator data were presented. In addition, the EGF-treated animals had histologic evidence of lung maturation as well as epithelial hyperplasia of the conducting airways, which was considered an effect of EGF administration. In a smaller number of lamb fetuses of comparable gestational age infused with EGF for 3½ days (approximately 8 μg/kg/d), Schellenberg *et al.* (22) did not observe any effects of EGF on lung distensibility or epithelial hyperplasia. However, Haigh *et al.* (3) injected 8 μg of human EGF into the peritoneum of fetal rabbits on d 25 of gestation and found increased surfactant phospholipids in tracheal lavage specimens obtained at delivery 3 d later. In a related study, Kennedy *et al.* (23) showed that infusion of 64 μg/kg EGF to fetal sheep at 132 d of gestation decreased lung liquid production by a mechanism that was not blocked by β-adrenergic antagonists. Decreased production of lung liquid is normally seen in preparation for birth (24).

We did not find a difference in lung compliance between EGF-treated and control animals. Our results are similar, in this regard, to those obtained from surfactant-treated human infants with RDS compared with untreated controls (25, 26). These failures to detect compliance differences may be due to collecting meas-

Table 4. *Comparison of proportion of parenchyma air space and tissue space, air space size as the mean linear intercept (MLI), and air space surface to volume ratio in lungs of EGF-treated (n = 4) and control (n = 4) rhesus infants**

	Parenchyma air space (%)	Parenchyma tissue space (%)	Air space size (MLI) (μm)	Surface to volume ratio
Control	67.4 ± 4.5	32.6 ± 4.5	92.3 ± 3.2	0.044 ± 0.001
EGF	85.3 ± 3.0†	14.7 ± 3.0†	98.9 ± 5.9	0.041 ± 0.002

* Values are means ± SEM.
† $p < 0.05$, for EGF group *vs* control group.

urements on ventilator breaths when there is lung overdistention as well as the inability to reduce lung stiffness by these therapies.

Our findings of increased surfactant apoprotein A and surfactant phospholipids in the amniotic fluid of the EGF-treated monkeys are consistent with a previous report from our center. Plopper *et al.* (4) observed cytodifferentiation of alveolar type II cells in the lungs of animals delivered at 128 d of gestation after fetal EGF exposure similar to that described in this report but killed at birth. There was marked loss of glycogen from these cells and a decrease in the number of type II cells without lamellar bodies. Their data suggest that increased surfactant production and secretion may be responsible for the functional maturation of lung that we observed after EGF exposure. In addition, a previous report with this rhesus model has shown maturation of the tracheal mucous secretory cells after fetal EGF exposure (27). However, the proliferation of basal and intermediate cells resulting in epithelial sloughs that Stahlman *et al.* (28) observed in the larger airways in fetal lambs exposed to EGF were not seen. Thus, there may be species differences in the pulmonary responses to EGF, particularly if there are critical times during gestation for EGF to produce its pulmonary effects or differences in the availability of vitamin A at such times.

We elected to provide broad exposure to EGF in this series of experiments, which were run in parallel to those described in the study by Plopper *et al.* (4), to determine whether any response to EGF would be detectable. We considered that intraperitoneal EGF could reach the lungs by the systemic route, whereas intraamniotic EGF could be inhaled during fetal breathing and reach the lung directly. In addition, EGF may have been absorbed directly into the fetal circulation after intraamniotic injection by the recently described intramembranous route (29, 30). This discovery has important implications for the pharmacologic treatment of the fetus (30). Of course, EGF could be swallowed and could have produced the significant increase in gut weight in the EGF-treated group. Experiments are in progress to determine the dose of EGF, the duration of exposure, and which route of exposure produced the observed effects on lung and gut maturation.

In vitro exposure to EGF has been reported to produce biochemical and histologic maturation in lung tissue of several species (1, 3). Gross *et al.* (1) showed a dose-dependent effect of EGF exposure on the rate of choline incorporation into phosphatidylcholine in explants of fetal rat lungs. Similarly, Haigh *et al.* (3) showed that human EGF enhanced the incorporation of radiolabeled choline into phosphatidylcholine in cultured fetal rabbit alveolar type II cells. Whitsett *et al.* (2) demonstrated that EGF exposure for 2 d induced the production of surfactant apoprotein A in explants of human lung tissue obtained at 15–24 wk of gestation. Preliminary work by Nielsen (31) suggested that the effect of EGF on epithelial cells might be indirect and mediated by interaction with fibroblasts. This was supported by the finding of Sen and Cake (32) that a fibroblast-derived factor stimulated EGF-induced choline incorporation into phosphatidylcholine by pure alveolar type II cells from rats. Cortisol was not required for this action, but the action was similar to findings of others on the mechanism of cortisol stimulation of type II cells (33, 34). In contrast, both Scott (35) and Raaberg *et al.* (36) were able to demonstrate direct EGF stimulation of type II cells without other cells or cell-conditioned media. The latter group also demonstrated EGF production within type II cells and suggested an autocrine function for this peptide growth factor. Thus, although the mechanism of action of EGF is incompletely understood, it does not appear that the effects of EGF on alveolar type II cells are mediated by cortisol.

Receptors for EGF and its fetal form, transforming growth factor-α, are widely distributed in fetal tissues including the lung and specifically the alveolar type II cells (37–39). This suggests that EGF/transforming growth factor-α plays a role in fetal development. In addition to the pulmonary maturational effects discussed above, we observed a trend toward decreased lung weight with EGF treatment. A similar decrease has been observed with glucocorticoid stimulation of fetal lung maturity (34). However, we observed an increase in adrenal and gut weights, whereas total body weight and placental weight were unaffected by EGF treatment. EGF has been reported to have similar effects on fetal sheep in addition to marked effects on their skin and wool (40). Interestingly, body growth of rats and mice can be either stimulated or retarded by EGF depending on their stage of development when EGF is administered (41, 42), and suppression of IGF has been observed during EGF-induced growth retardation (43). Our results on brain weights in fetal monkeys are consistent with an apparent lack of EGF effect on brain growth (44). Our results after 1 wk of EGF exposure do not allow speculation on whether longer exposure of fetal macaques would affect ponderal growth or lung growth.

Biochemical, histologic, and clinical studies have established that rhesus infants delivered near 80% of term can provide useful models of the RDS (8–10). However, previous studies were terminated 2–24 h after birth (10–12) and the complete time course of respiratory distress in this species had not been established before the studies described herein. The clinical findings for our control animals confirm that rhesus infants delivered at 78% of gestation provide an acceptable primate model for the entire acute course of RDS when intensive care support is provided.

RDS continues to be a major cause of neonatal mortality and chronic lung disease continues to occur in survivors in spite of advances in treatment, including prenatal treatment with glucocorticoids and postnatal treatment with exogenous surfactant. Both surfactant deficiency and structural immaturity are important factors in determining these outcomes (45, 46). In our study, rhesus infants treated with EGF had advancement of both biochemical and structural development of their lungs and would be predicted to have both lower mortality and a lower incidence of BPD than the control infants. Although BPD has been described in nonhuman primate infants after RDS (47), our experiments were not designed to detect an effect on BPD. However, our findings do have implications for the prevention of RDS in human premature infants and may be even more significant if a role for reducing the incidence of BPD is confirmed.

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