

# rhIGF-1/BP3 Preserves Lung Growth and Prevents Pulmonary Hypertension in Experimental Bronchopulmonary Dysplasia

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## Abstract

**Rationale:** Antenatal factors, such as chorioamnionitis, preeclampsia, and postnatal injury, are associated with an increased risk for bronchopulmonary dysplasia (BPD) and pulmonary hypertension (PH) after preterm birth. IGF-1 (insulin-like growth factor-1) is markedly decreased in normal preterm infants, but whether IGF-1 treatment can prevent BPD or PH is unknown.

**Objectives:** To evaluate whether postnatal treatment with rhIGF-1 (recombinant human IGF-1)/BP3 (binding peptide 3) improves lung growth and prevents PH in two antenatal models of BPD induced by intraamniotic exposure to endotoxin (ETX) or sFlt-1 (soluble fms-like tyrosine kinase 1), and in a postnatal model due to prolonged hyperoxia.

**Methods:** ETX or sFlt-1 were administered into the amniotic sac of pregnant rats at Embryonic Day 20 to simulate antenatal models of chorioamnionitis and preeclampsia, respectively. Pups were delivered by cesarean section at Embryonic Day 22 and treated with rhIGF-1/BP3 (0.02–20 mg/kg/d intraperitoneal) or buffer for

2 weeks. Study endpoints included radial alveolar counts (RACs), vessel density, and right ventricular hypertrophy (RVH). Direct effects of rhIGF-1/BP3 (250 ng/ml) on fetal lung endothelial cell proliferation and tube formation and alveolar type 2 cell proliferation were studied by standard methods *in vitro*.

**Measurements and Main Results:** Antenatal ETX and antenatal sFlt-1 reduced RAC and decreased RVH in infant rats. In both models, postnatal rhIGF-1/BP3 treatment restored RAC and RVH to normal values when compared with placebo injections. rhIGF-1/BP3 treatment also preserved lung structure and prevented RVH after postnatal hyperoxia. *In vitro* studies showed that rhIGF-1/BP3 treatment increased lung endothelial cell and alveolar type 2 cell proliferation.

**Conclusions:** Postnatal rhIGF-1/BP3 treatment preserved lung structure and prevented RVH in antenatal and postnatal BPD models. rhIGF-1/BP3 treatment may provide a novel strategy for the prevention of BPD in preterm infants.

**Keywords:** insulin-like growth factor-1; angiogenesis; lung development; prematurity; pulmonary hypertension

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## At a Glance Commentary

### Scientific Knowledge on the

**Subject:** Antenatal determinants, including chorioamnionitis, preeclampsia, and postnatal hyperoxia, contribute to the development of bronchopulmonary dysplasia (BPD) and pulmonary hypertension (PH) after preterm birth, but therapies that prevent BPD and PH are lacking.

### What This Study Adds to the Field:

We found that early treatment with IGF-1 (recombinant human insulin-like growth factor-1)/BP3 preserves lung growth and maintains lung function in infant rats after exposure to antenatal stresses, such as endotoxin and sFlt-1 (soluble fms-like tyrosine kinase 1), as well as postnatal hyperoxia. These findings support the speculation that IGF-1 treatment may prevent BPD and PH in human infants.

Despite improvements in care, preterm children remain at high risk for mortality and respiratory morbidities due to the development of bronchopulmonary dysplasia (BPD) (1). BPD is the chronic lung disease of prematurity that develops in infants who require respiratory support at birth because of immaturity of the preterm lung. BPD occurs in over 40% of infants born below 29 weeks gestation, leading to roughly 15,000 new cases in the United States each year (1–4). BPD is strongly associated with significant complications, including the prolonged need for mechanical ventilation, respiratory support and oxygen therapy, longer duration of hospitalization, and higher rates of late respiratory disease and nonrespiratory comorbidities (5, 6). Sustained abnormalities of lung function, poor exercise tolerance, and the need for chronic respiratory medications throughout childhood and adolescence are also increased in former preterm infants (7–11).

Advances in postnatal care have increased survival of extremely preterm infants, but the incidence of BPD has not changed over the past few decades (4), which may contribute to greater numbers of extremely preterm infants who are at highest risk for severe BPD (2, 5). Although postnatal factors, such as hyperoxia, mechanical ventilation, and others,

contribute to the risk for BPD, epidemiologic studies have further identified important roles for antenatal factors in disease pathogenesis (9, 11–21). Recent findings emphasize antenatal factors that are independent of postnatal injury as major determinants of risk for BPD and late respiratory outcomes (20, 21). An NHLBI-sponsored workshop discussed the importance of prenatal influences on lung growth and development and subsequent respiratory function after preterm birth that can persist throughout childhood (11).

Antenatal factors, such as chorioamnionitis (CA), preeclampsia (PE), and others, are strongly associated with an increased risk for BPD, especially when associated with intrauterine growth restriction (IUGR) as a biomarker for severe placental dysfunction and fetal stress (15, 22–29). Early changes in circulating angiogenic peptides, including decreased proangiogenic factors, increased antiangiogenic factors (including sFlt-1 [soluble fms-like tyrosine kinase 1], an endogenous vascular endothelial growth factor inhibitor that is markedly increased in the blood and amniotic fluid of women with PE), and decreased endothelial progenitor cells, are associated with both abnormal placental vascular disease and high risk for BPD and pulmonary hypertension (PH) (27–32). These data support the hypotheses that antenatal mechanisms that promote an antiangiogenic fetal environment contribute to high risk for BPD and PH in preterm infants and suggest novel targets for disease prevention.

Although mechanisms linking placental dysfunction with BPD risk are incompletely understood, early disruption of critical signaling pathways, including IGF-1 (insulin-like growth factor-1), which has strong angiogenic activity, have been implicated in the etiology of BPD (32). IGF-1 plays a key role in normal lung development *in utero* and impaired IGF-1 signaling contributes to disease pathobiology in experimental models of acute lung injury and PH (33–36). Importantly, low IGF-1 levels are also strongly associated with IUGR and PE (31) and persist after preterm birth (37), but whether treatment with IGF-1 can improve respiratory outcomes after preterm birth and prevent BPD and PH is unknown.

Thus, the need for preventive strategies remains a major challenge to better improve long-term respiratory

outcomes after preterm birth. Based on the important role of IGF-1 in lung development and reports that low IGF levels shortly after birth are associated with the subsequent development of BPD, we propose the hypothesis that postnatal treatment with rhIGF-1 (recombinant human IGF-1)/BP3 (binding peptide 3) would enhance lung alveolar and vascular growth and lung function and prevent the development of PH in experimental BPD as induced by antenatal stress.

To test this hypothesis, we performed a series of studies using two models of BPD: one model that is associated with CA as induced by intraamniotic exposure to endotoxin (ETX) (38) and the second model that mimics the effects of PE, which is induced by intraamniotic injection of sFlt-1 (39, 40). We then tested the hypothesis that IGF-1 therapy could preserve lung growth and function in a traditional postnatal model of BPD due to hyperoxia exposure. We report that postnatal therapy with rhIGF-1/BP3 improves lung structure and function and prevents PH in each of these animal models, suggesting that IGF-1 may provide a novel strategy for the prevention of BPD.

## Methods

### General Approach

Based on strong epidemiologic and clinical data demonstrating the critical role of antenatal determinants in the pathogenesis of BPD in preterm infants (20, 21), we studied the impact of rhIGF-1/BP3 therapy on lung alveolar and vascular growth and lung function. In this study, we used rodent models of CA as induced by intraamniotic ETX injection and PE as mimicked by sFlt-1 injection, as previously described (38–40) (Figure 1). To further determine whether rhIGF-1/BP3 therapy could improve lung structure and function induced by postnatal hyperoxia exposure, we also studied the effects of rhIGF-1/BP3 in a long-standing model of the “old BPD,” which reflects the adverse effects of postnatal interventions, such as hyperoxia (1, 41), without consideration of the important roles of antenatal determinants in BPD pathobiology of the modern era (2–4, 20, 21, 42, 43). All procedures and protocols were approved by the Animal Care and Use Committee at the University of Colorado Health Sciences Center. See the



rhIGF-1/BP3 treatment were performed to explore whether early intervention to accelerate postnatal adaptation of the lung during hyperoxia would be sufficient to provide sustained improvement in lung growth.

### Study Measurements

**Lung function.** Lung function was determined in 14-day old pups with the flexiVent system (SCIREQ), as previously described (40).

**TISSUE FOR HISTOLOGICAL ANALYSIS.** Lungs were inflated with 4% paraformaldehyde and maintained at 20 cm H<sub>2</sub>O pressure for overnight fixation (38–40). A 2-mm-thick transverse section was taken from the midplanes of the right lower and left lobes of the fixed lungs per animal to process and embed in paraffin wax.

**Immunohistochemistry.** Slides with 5- $\mu$ m paraffin sections were stained

with hematoxylin and eosin for assessment of alveolar structure, and with von Willebrand factor, an endothelial cell-specific marker, for assessment of vascular density.

**MORPHOMETRIC ANALYSIS.** Radial alveolar counts (RACs) and pulmonary vessel density were determined by standard morphometric techniques (38–41, 45, 46).

**INDICES OF RVH.** The right ventricle (RV) and left ventricle plus septum were dissected and weighed. The ratios of RV to left ventricle plus septum weights and RV/body weights were determined to evaluate RVH.

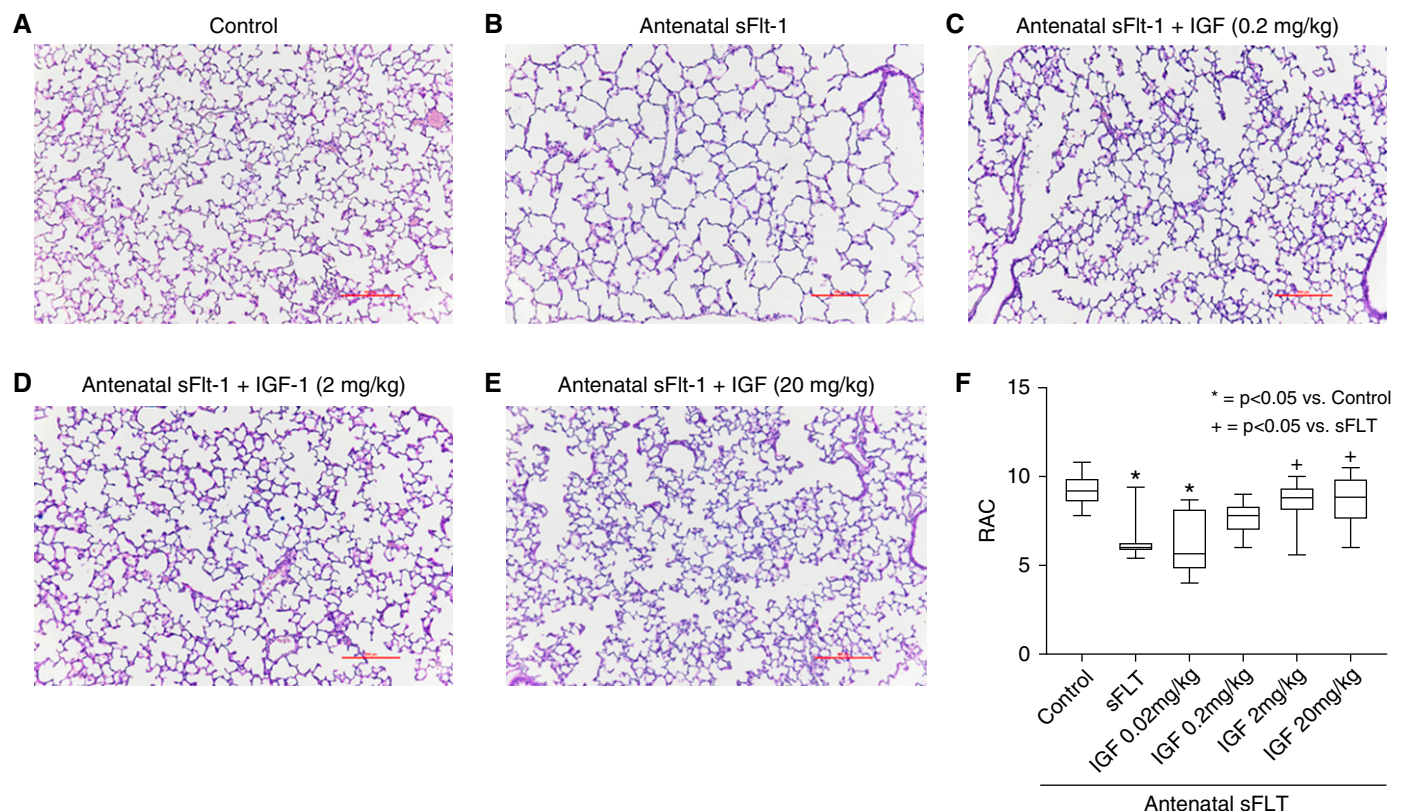
**WESTERN BLOT ANALYSIS.** Proteins were obtained from rapidly frozen lung samples for Western blot analysis of IGF-1 and endothelial nitric oxide synthase (eNOS) per standard methods (see online supplement).

**RNA ISOLATION AND MICROARRAY ANALYSIS.** The whole lungs of offspring from intraamniotic ETX exposed and control pups were harvested on Day 0 of life ( $n = 4$

for each group). Total RNA was extracted using the miRNeasy Mini Kit (Qiagen) per manufacturer's instructions. Following isolation, total RNA was quantified using a NanoDrop ND-1000 spectrophotometer (Thermo Scientific). RNA from these samples were analyzed by standard procedures using Affymetric microarrays (Clariom D Rat Transcriptome Array 1.0). Additional details are provided in the online supplement.

### Isolated Neonatal Lung Endothelial Cells and Alveolar Type 2 Cells

Lung endothelial cells (LECs) and alveolar type 2 cells (AT2Cs) were collected from neonatal lungs and studied *in vitro* by standard methods. We also studied the effects of antenatal ETX exposure on LEC and AT2C growth, and the *in vitro* response to IGF-1 treatment.



**Figure 2.** Effects of rhIGF-1 (recombinant human insulin-like growth factor-1)/BP3 (binding peptide 3) treatment on distal lung structure in infant rats after antenatal intraamniotic injection of sFlt-1 (soluble fms-like tyrosine kinase 1) in experimental preeclampsia. (A and B) In comparison with saline control rats (A), prenatal exposure to sFlt-1 causes lung simplification (B). (C–E) As shown, postnatal IGF-1 treatment causes dose-related improvement in lung histology (C–E), as quantified by changes in RAC (F). Micrographs are representative and were obtained at the same magnification (scale bars, 100  $\mu$ m; box plots represent median, interquartile range, and minimum–maximum data points). Animal numbers per study group: control rats = 20; sFlt-1 = 18; rhIGF-1/BP3 0.02 mg/kg = 10; 0.2 mg/kg = 20; 2 mg/kg = 20; and 20 mg/kg = 10. RAC = radial alveolar count.

## Statistical Analyses

Statistical analysis was performed with the GraphPad Prism 8.0 software package (GraphPad Software). Statistical comparisons were made between groups using the *t* test or ANOVA with Kruskal-Wallis and Dunn's *post hoc* analysis for significance.  $P < 0.05$  was considered significant. Data are presented as interquartile range with minimum–maximum data points.

## Results

### Effects of Postnatal rhIGF-1/BP3 Treatment after Antenatal sFlt-1 Exposure

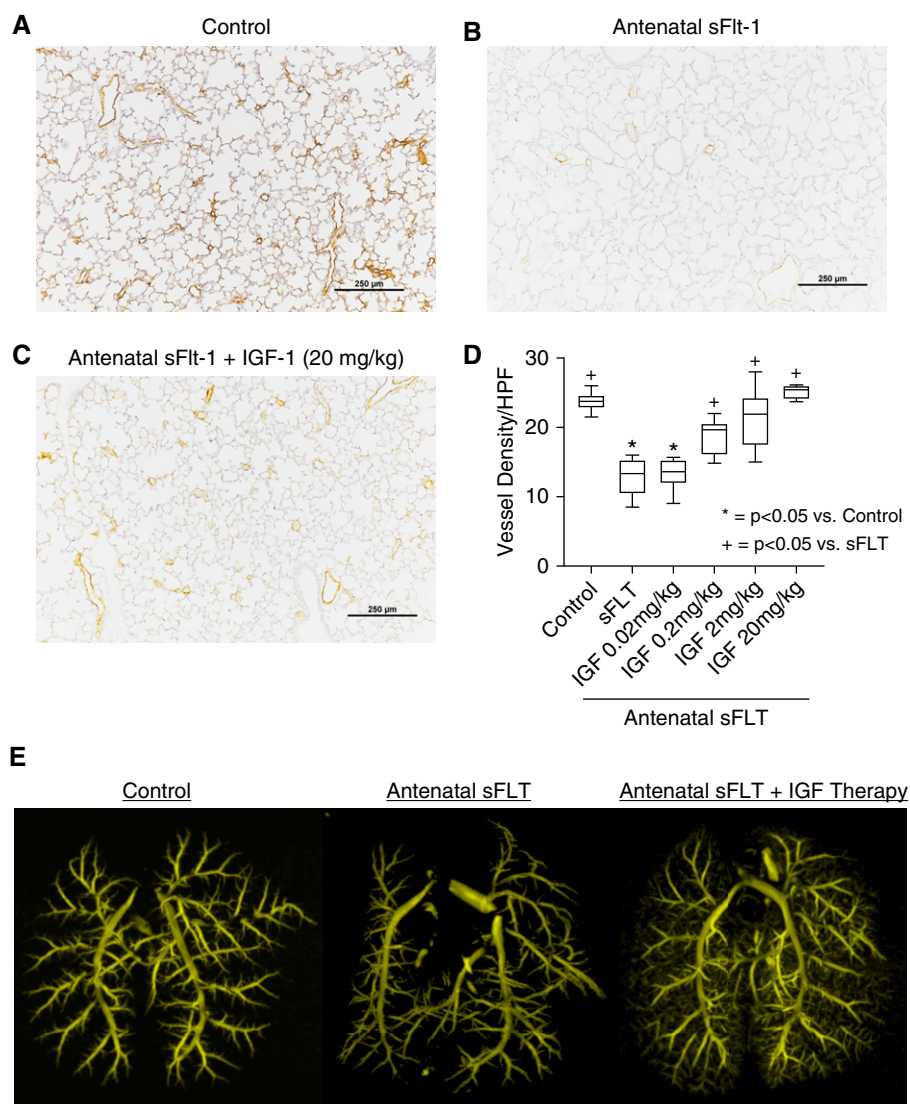
Antenatal injection of sFlt-1 caused distal lung simplification in infant rats, as evident from increased distal airspace size and reduced RAC (Figure 2). Birth weights were not different between sFlt-1–treated and control pups. In comparison with saline control rats, prenatal exposure to sFlt-1 reduced RAC by  $32\% \pm 1$  ( $P < 0.0001$ ). rhIGF-1/BP3 treatment caused dose-related improvement in lung histology, as quantified by increases in infant lung RAC to values that were not different from normal control rats (see Figure 2F). Lung histology and vessel counts show that after antenatal sFlt-1 exposure vascular density in the distal lung was reduced by  $42\% \pm 3$  ( $P < 0.0038$ ) when compared with untreated control rats (Figure 3). Postnatal rhIGF-1/BP3 treatment caused dose-related increases in vessel density after antenatal sFlt-1 treatment, restoring vascular growth to levels observed in control 14-day-old infant rats. Angiograms showed enhanced filling of distal vascular branches in infant rats after rhIGF-1/BP3 treatment (see Figure 3E). In addition to structural improvement in distal lung airspace and vascular growth, we found that rhIGF-1/BP3 treatment reduced RVH and improved lung function in infant rats at Day 14 that had been exposed to antenatal sFlt-1 (Figure 4). Additional analysis of these data found no differences in the severity of impairment of lung structure or function, severity of RVH in response to antenatal sFlt-1, or differences in responsiveness to rhIGF-1/BP3 treatment according to sex.

### Effects of Postnatal rhIGF-1/BP3 Treatment after Antenatal ETX Exposure

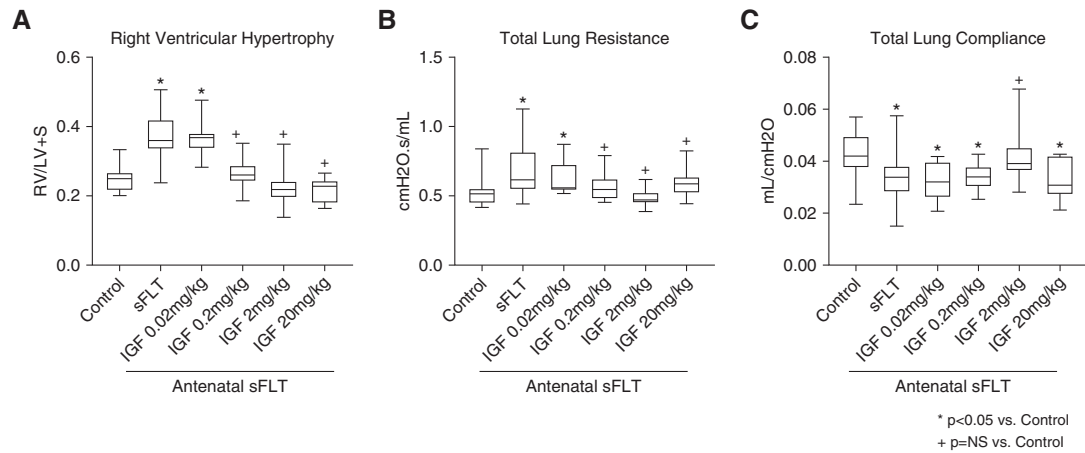
As observed after antenatal sFlt-1, intraamniotic ETX injection to induce an

inflammatory model of CA decreased distal lung growth, as evident from the reduction in RAC by  $39 \pm 1\%$  at 2 weeks of age when compared with vehicle-treated saline control rats ( $P < 0.0001$ ) (Figure 5). Birth weights were not different between ETX-treated and control pups. Postnatal treatment with rhIGF-1/BP3 causes dose-related improvements in lung structure, resulting in similar RAC as observed in

unexposed control rats. Vessel density at 2 weeks was reduced by 36% in pups that had been exposed to antenatal ETX, which was restored to normal values by postnatal rhIGF-1/BP3 treatment (Figure 6). rhIGF-1/BP3 treatment improved lung vascular density in 2-week-old infant rats after antenatal ETX injection in experimental CA (see Figure 6). Lung histology and vessel counts after immunostaining of endothelial



**Figure 3.** Effects of rhIGF-1 (recombinant human insulin-like growth factor-1)/BP3 (binding peptide 3) treatment on lung vascular density in infant rats after antenatal intraamniotic injection of sFlt-1 (soluble fms-like tyrosine kinase 1) in experimental preeclampsia. (A–D) Lung histology and vessel counts after immunostaining of endothelial cells with von Willebrand factor show that sFlt-1–exposed rats have decreased vascular density when compared with control rats (A and B) and that IGF-1 treatment restores vessel density at 14 days (C and D) (scale bars, 250  $\mu$ m). (E) Angiograms after barium vascular injection illustrates improved distal branching in infant rats after IGF-1 treatment. Box plots represent median, interquartile range, and minimum–maximum data points. Animal numbers per study group: control rats = 10; sFlt-1 = 10; rhIGF-1/BP3 0.02 mg/kg = 10; 0.2 mg/kg = 10; 2 mg/kg = 10; and 20 mg/kg = 10. HPF = high-powered field.



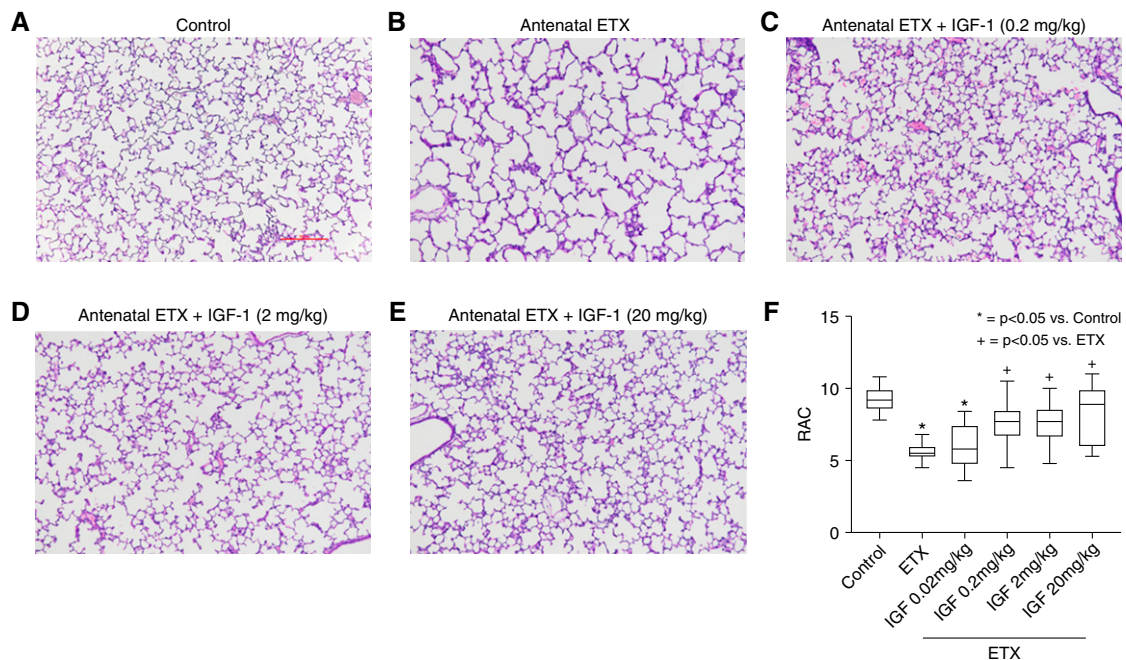
**Figure 4.** (A–C) Effects of rhIGF-1 (recombinant human insulin-like growth factor-1)/BP3 (binding peptide 3) treatment on RV hypertrophy (A) and lung function (B and C) in infant rats after antenatal intraamniotic injection of sFlt-1 (soluble fms-like tyrosine kinase 1) in experimental preeclampsia. (B and C) As shown, IGF-1 treatment causes dose-related improvement in RV hypertrophy, total respiratory system resistance (B), and compliance (C), with the exception of lung compliance as measured in the IGF-1 20 mg/kg group. Box plots represent median, interquartile range, and minimum–maximum data points. Animal numbers per study group: control rats = 19; sFlt-1 = 18; rhIGF-1/BP3 0.02 mg/kg = 10; 0.2 mg/kg = 20; 2 mg/kg = 20; and 20 mg/kg = 10. LV+S = left ventricle plus septum; NS = not significant; RV = right ventricle.

cells show that ETX-exposed rats had decreased vascular density when compared with control rats and that rhIGF-1/BP3 treatment restored vessel density to normal values. rhIGF-1/BP3 treatment prevented RVH and improved total

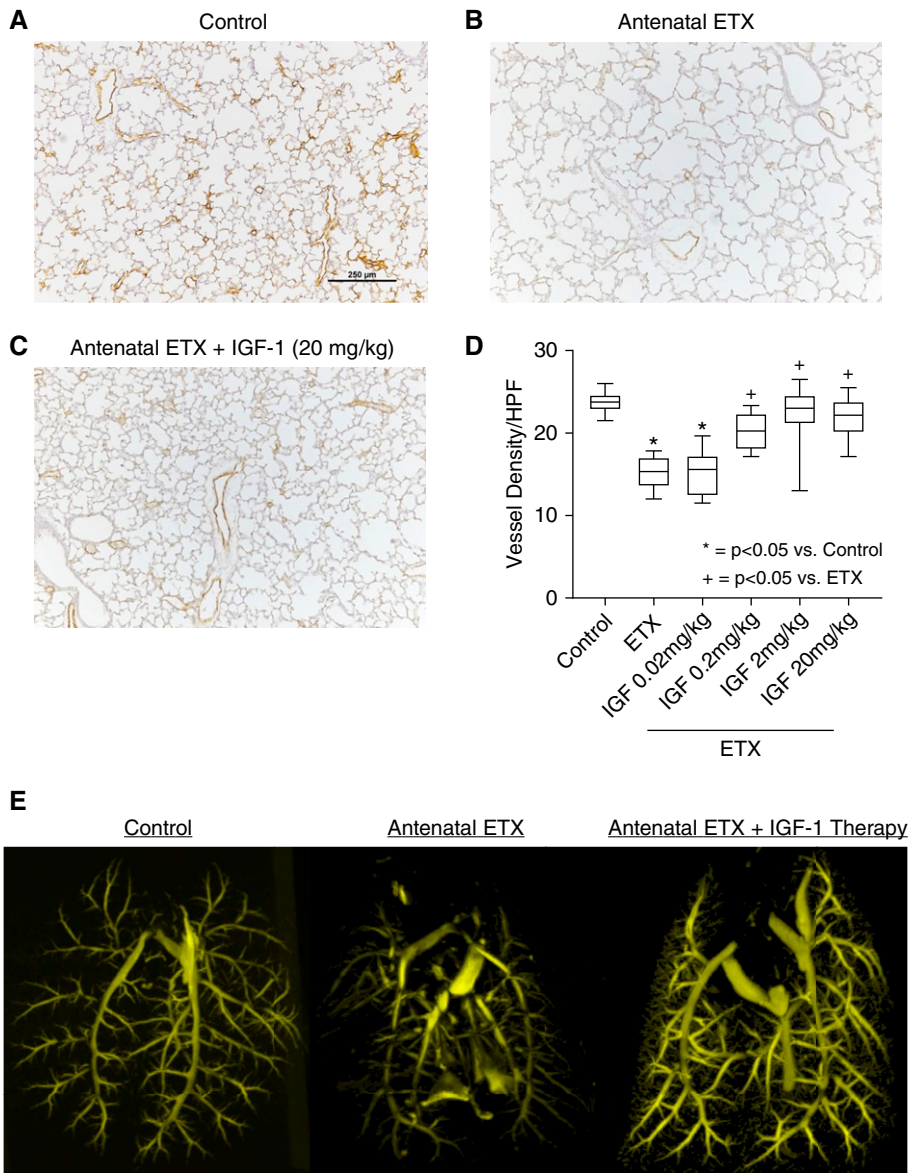
respiratory system compliance and resistance in infant rats after antenatal ETX injection (Figure 7). Additional analysis of these data found no differences in the severity of impairment of lung structure or function, severity of RVH in response to

antenatal ETX, or differences in responsiveness to rhIGF-1/BP3 treatment according to sex.

We sought to determine whether shorter periods of therapy would be sufficient to improve distal lung



**Figure 5.** Effects of rhIGF-1 (recombinant human insulin-like growth factor-1)/BP3 (binding peptide 3) treatment on distal lung structure in infant rats after antenatal intraamniotic injection of endotoxin (ETX) in experimental chorioamnionitis. (A and B) Prenatal exposure to ETX decreases septation when compared with saline control rats. (C–F) Postnatal treatment with IGF-1 causes dose-related improvements in lung structure (C–E) and RAC (F). Micrographs are representative and were obtained at the same magnification (scale bars, 100  $\mu$ m; box plots represent median, interquartile range, and minimum–maximum data points). Animal numbers per study group: control rats = 20; ETX = 19; rhIGF-1/BP3 0.02 mg/kg = 10; 0.2 mg/kg = 18; 2 mg/kg = 19; and 20 mg/kg = 8. RAC = radial alveolar count.



**Figure 6.** Effects of rhIGF-1 (recombinant human insulin-like growth factor-1)/BP3 (binding peptide 3) treatment on lung vascular density in 2-week-old infant rats after antenatal endotoxin (ETX) injection in experimental chorioamnionitis. (A–D) Lung histology and vessel counts after immunostaining of endothelial cells with von Willebrand factor show that ETX-exposed rats have decreased vascular density when compared with control rats (A and B) and that rhIGF-1/BP3 treatment restores vessel density (C and D). (E) Angiograms after barium vascular injection illustrates improved distal branching in infant rats after rhIGF-1/BP3 treatment. Box plots represent median, interquartile range, and minimum–maximum data points. Scale bar, 250  $\mu$ m. Animal numbers per study group: control rats = 10; ETX = 10; rhIGF-1/BP3 0.02 mg/kg = 10; 0.2 mg/kg = 10; 2 mg/kg = 10; and 20 mg/kg = 10. HPF = high-powered field.

structure and vessel density, and we treated rat pups with rhIGF-1/BP3 (20 mg/kg) during the first 3 days of life and assessed lung structure at 14 days (Figure 8). As shown, 3 days of treatment improved RAC and vessel density in infant lungs, suggesting that shorter duration of treatment may be sufficient in this

model. To further explore mechanisms related to the effects of rhIGF-1/BP3 treatment on lung structure in these models, we studied the effects of rhIGF-1/BP3 treatment on lung IGF-1 (Figure 9) and eNOS protein contents at 2 weeks of age after intraamniotic ETX or sFlt-1 exposure. In comparison with

untreated rats, rhIGF-1/BP3 infusions increased lung IGF and eNOS content at Day 14.

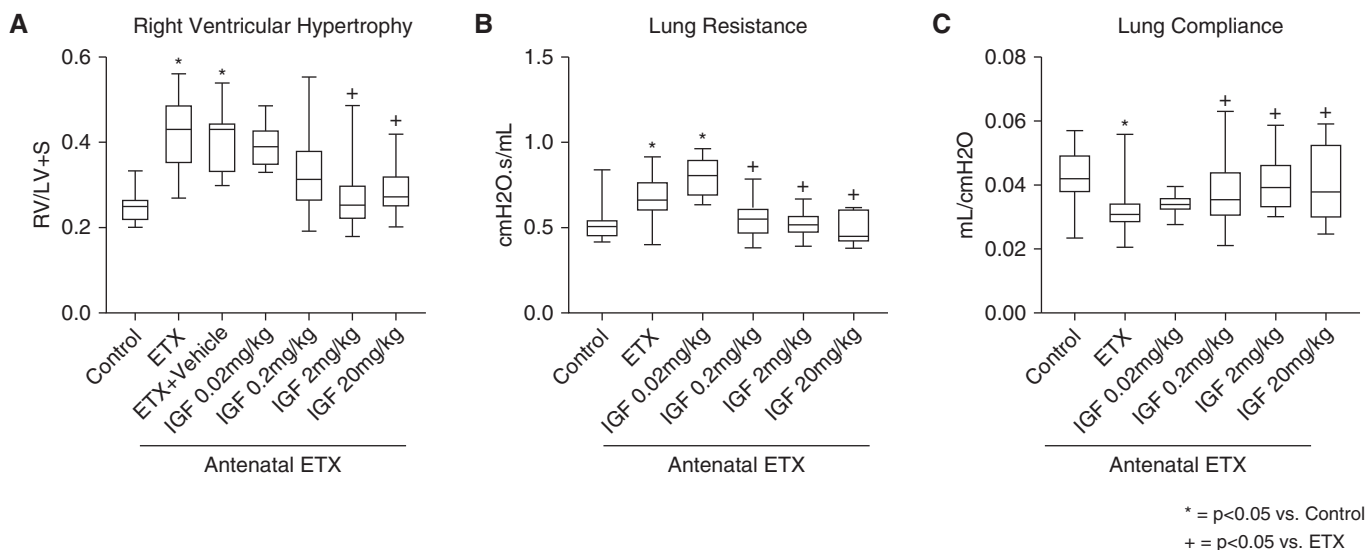
### Effects of Postnatal rhIGF-1/BP3 Treatment after Prolonged Hyperoxia Exposure

As noted, rhIGF-1/BP3 treatment preserved lung structure and function and prevented RVH in two distinct models of antenatal stress that mimic CA and PE. To determine whether rhIGF-1/BP3 treatment would also be effective in a more traditional BPD model induced by postnatal hyperoxia, we treated rats with rhIGF-1/BP3 during exposure to 90%  $F_{I_{O_2}}$  for 2 weeks and assessed lung structure and function for comparisons (Figure 10). rhIGF-1/BP3 treatment prevented the adverse effects of hyperoxia when administered throughout the study period; however, the beneficial effects of rhIGF-1/BP3 treatment were not observed if treatment was halted after only 3 days (see Figure 10 and Figure 11). There were no differences in the severity of impairment of lung structure or function, or severity of RVH, in response to hyperoxia, or differences in responsiveness to rhIGF-1/BP3 treatment as based on sex.

We also examined dose-related changes in serum IGF-1 levels in rats 60 minutes after rhIGF-1/BP3 infusion in pups from each of the models, including those exposed to sFlt-1, ETX, or postnatal hyperoxia. In each study protocol, rhIGF-1/BP3 infusion increased serum IGF-1 levels in dose-related fashion (Figure 12).

### Effects of rhIGF-1/BP3 Treatment on Fetal LECs and AT2Cs *In Vitro*

To explore potential mechanisms underlying the effects of rhIGF-1/BP3 treatment *in vivo*, we studied the direct effects of rhIGF-1/BP3 on isolated LEC and AT2C growth *in vitro*. (Figure 13) As shown, rhIGF-1/BP3 enhanced LEC growth and tube formation in dose-related fashion and directly stimulated neonatal rat AT2C growth (see Figure 13C). LECs and AT2Cs that were harvested after antenatal ETX exposure had impaired growth in comparison with control rats but were also responsive to exogenous rhIGF-1/BP3 exposure.



**Figure 7.** (A–C) Effects of IGF-1 (insulin-like growth factor-1) treatment on RV hypertrophy (A) and lung function (B and C) in infant rats after antenatal endotoxin (ETX) injection in experimental chorioamnionitis. rhIGF-1 (recombinant human IGF-1)/BP3 (binding peptide 3) treatment causes dose-related improvement in RV hypertrophy, total respiratory system resistance (B), and compliance (C). Box plots represent median, interquartile range, and minimum–maximum data points. Animal numbers per study group: control rats = 20; ETX = 19; rhIGF-1/BP3 0.02 mg/kg = 10; 0.2 mg/kg = 18; 2 mg/kg = 19; and 20 mg/kg = 8. LV+S = left ventricle plus septum; RV = right ventricle.

## Discussion

Since its original description over 50 years ago, there has been a growing awareness of the impact of antenatal determinants as key etiologic mechanisms of impaired lung growth that contribute to the persistently high incidence of BPD (9, 11, 15–23). In this study, we show that rhIGF-1/BP3 therapy preserves lung structure and function and prevents RVH in infant rat pups after exposure to two experimental models of antenatal stress that impair lung development, which include intraamniotic injections of ETX or sFlt-1 to mimic key pathogenetic features of CA and PE, respectively. We further found that in the standard postnatal model of hyperoxia-induced BPD, rhIGF-1/BP3 therapy also enhances lung growth and function and prevents RVH in infant rats. We show that rhIGF-1/BP3 treatment upregulates eNOS and directly stimulates neonatal LEC proliferation and tube formation, reflecting its proangiogenic properties. rhIGF-1/BP3 treatment also enhances AT2C growth, suggesting at least two mechanisms that may contribute to the effectiveness of rhIGF-1/BP3 in each of these animal models. Thus, in both antenatal models and in the postnatal hyperoxia model of BPD, rhIGF-1/BP3 therapy has demonstrable beneficial effects

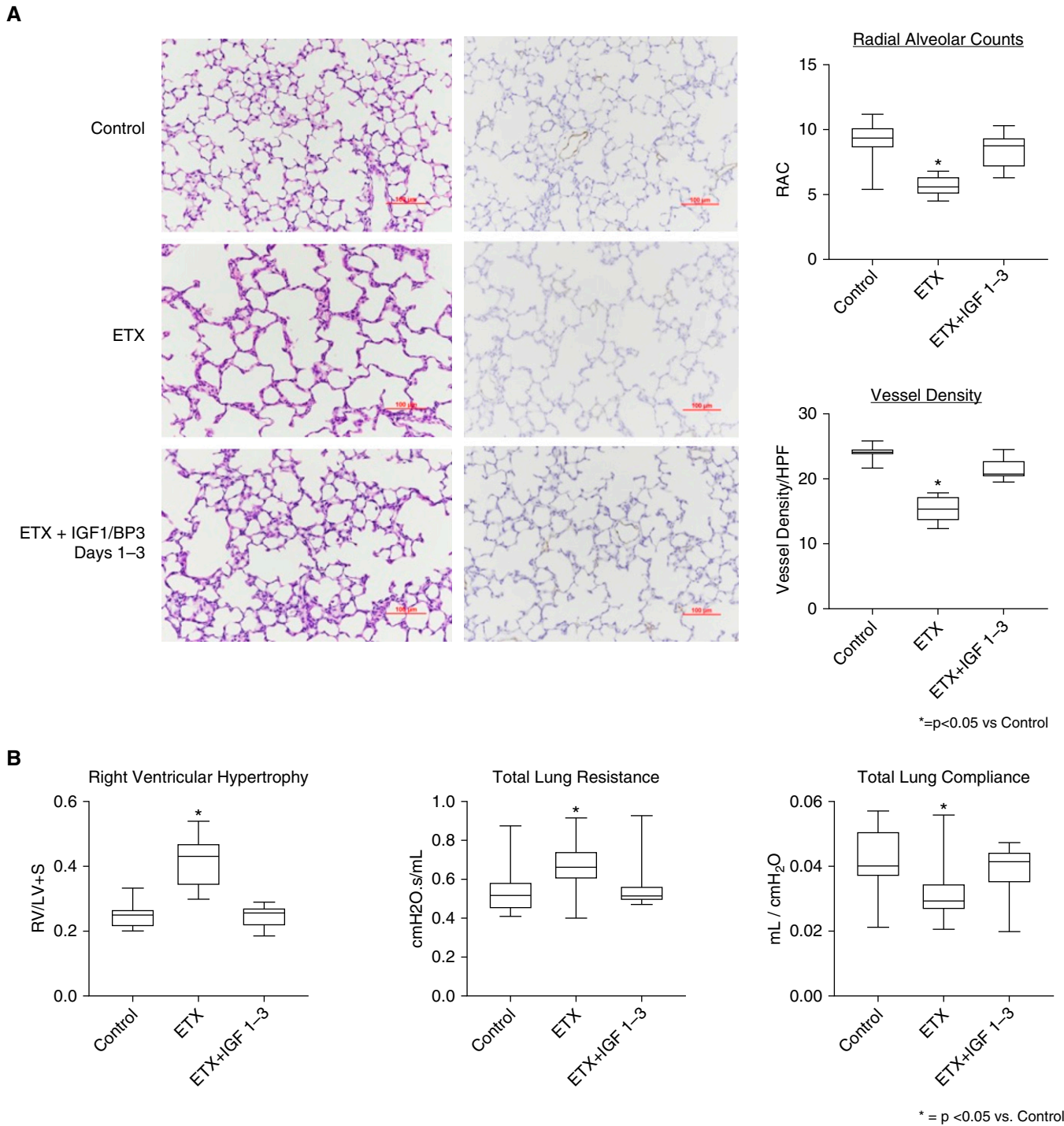
on enhancing lung growth, normalizing lung function, and preventing RVH in infant rats.

Although the pathogenesis of BPD is multifactorial and disease severity is modulated by the adverse effects of postnatal exposures, such as hyperoxia, mechanical ventilation, and other factors, strong experimental and clinical data show that antenatal events are key determinants of high risk for the “new BPD” independent of postnatal injury alone (20, 21, 26, 42). These clinical findings are supported by preclinical studies in which prenatal insults, even in the absence of postnatal hyperoxia or other injuries, are sufficient to cause sustained impairment of lung structure and function and RVH (38–40). As a result, strategies to reduce the incidence and severity of BPD will likely require early identification of at-risk infants with the application of early therapeutic interventions shortly after birth. The animal models used in this study address the need to examine pathogenetic mechanisms through which antenatal stress contribute to disease pathogenesis underlying the new BPD. (42, 43) The new BPD has been characterized by greater recognition of the impact of adverse fetal events in the pathobiology of BPD

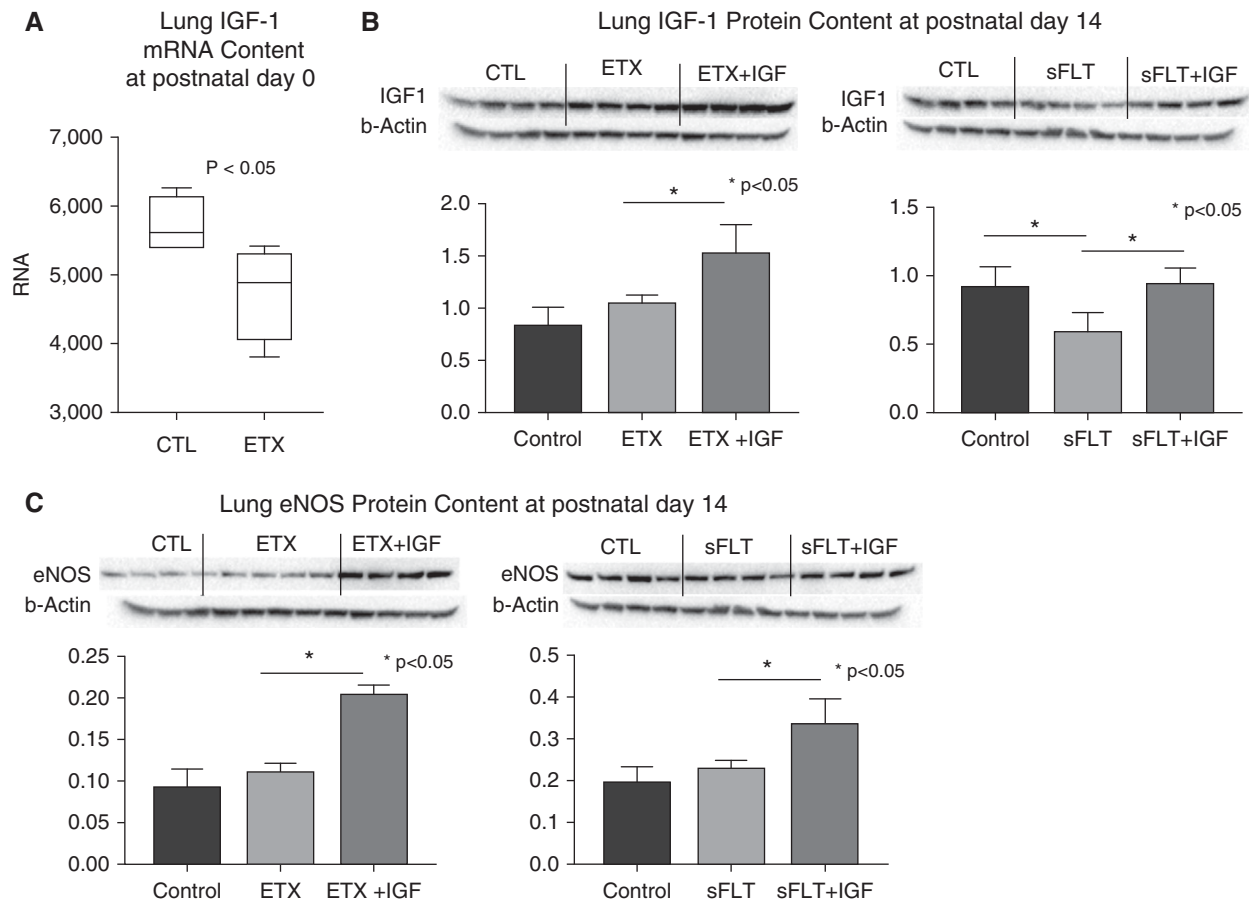
and late respiratory outcomes, as reflected by recent prospective studies (20, 21).

Several studies have shown a greater risk for BPD in preterm infants with IUGR, a key marker for adverse antenatal conditions strongly linked with placental dysfunction. A recent study reported a roughly twofold increase in early (28 d) and late (36 wk postmenstrual age) mortality, and the risk for BPD in IUGR infants born at or below 32 weeks gestation (24). Additionally, preterm infants who were born with IUGR remain at high risk for late respiratory morbidities and lung function at school age (22–25). When studied at a mean age of 11 years, children who were born prematurely with IUGR had lower lung function, including FEV<sub>1</sub> and diffusion capacity, suggesting abnormal airway function and decreased lung surface area, in comparison with non-IUGR prematurely born children (24). Although IUGR itself does not sufficiently provide mechanistic understanding of BPD risk *per se*, IUGR serves as a useful biomarker that strongly supports the hypothesis that antenatal events play critical roles in BPD etiology and long-term respiratory outcomes (25). Based on these changes in the features and persistent challenges of BPD, more studies that incorporate antenatal models that lead to impaired lung development will provide





**Figure 8.** Effects of short duration of postnatal rhIGF-1 (recombinant human insulin-like growth factor-1)/BP3 (binding peptide 3) treatment after exposure to antenatal endotoxin (ETX) on lung structure in infant rat lungs at 14 days of life. (A and B) As shown, 3 days of treatment improved RAC and vessel density in infant lungs (A) and rhIGF-1/BP3 treatment for 3 days improved right ventricular hypertrophy and lung mechanics at Day 14 in comparison with control rats who received antenatal ETX only (B). Box plots represent median, interquartile range, and minimum–maximum data points. Scale bars, 100  $\mu$ m. Animal numbers per study group: control rats = 10; ETX = 10; and rhIGF-BP3 Days 1 to 3, 2 mg/kg = 10. HPF = high-powered field; LV+S = left ventricle plus septum; RAC = radial alveolar count; RV = right ventricle.



**Figure 9.** Effects of IGF-1 (insulin-like growth factor-1) treatment on lung IGF-1 mRNA (A) and protein (B) and lung eNOS (endothelial nitric oxide synthase) protein (C) contents at 2 weeks of age after intraamniotic ETX or sFlt-1 (soluble fms-like tyrosine kinase 1) exposure. In comparison with untreated rats, rhIGF-1 (recombinant human IGF-1)/BP3 (binding peptide 3) infusions increased lung IGF and eNOS content in treated animals. Representative blots are shown to represent each study group, with 4 animals per group. Box plots represent median, interquartile range, and minimum–maximum data points. Bars represent mean  $\pm$  SEM. CTL=control; ETX=endotoxin.

insights into disease pathobiology and develop potential preventive strategies.

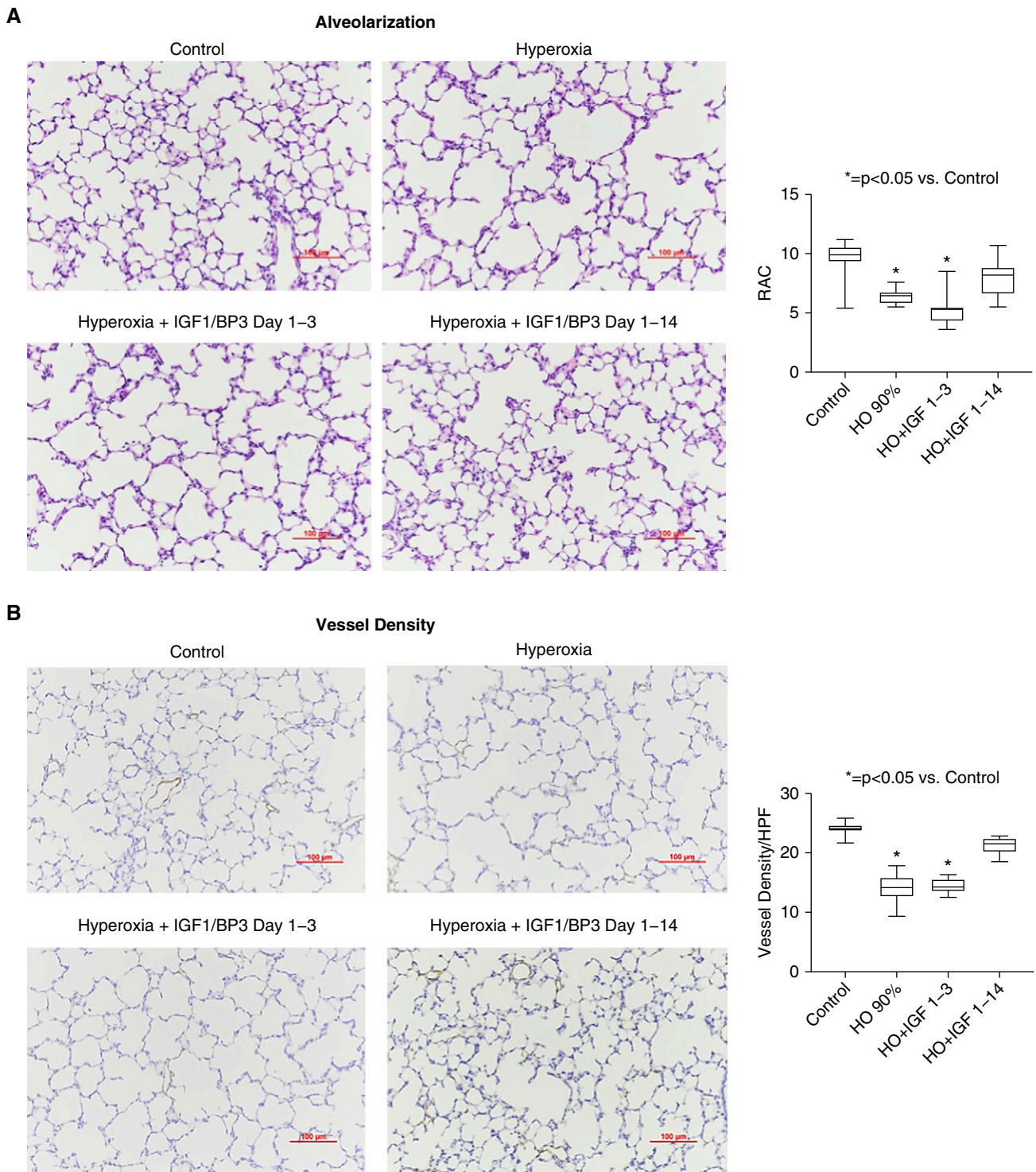
The presence of placental vascular abnormalities with preterm birth are strongly associated with the subsequent risk for BPD and BPD with PH (27–29). Specific mechanisms through which placental dysfunction disrupts lung development and contributes to BPD and PH are uncertain. Preclinical studies suggest that disruption of angiogenesis due to adverse antenatal factors, such as CA, PE, maternal smoking, and postnatal injury after preterm birth, can cause pulmonary vascular disease that not only leads to PH but can also impair distal lung growth. Laboratory studies have shown that the developing endothelial cell plays a key role in regulation and coordination of epithelial growth and distal airspace structure through the production of critical “angiocrines,” such as nitric

oxide, hepatocyte growth factor, vitamin A, IGF-1, and others (47–49).

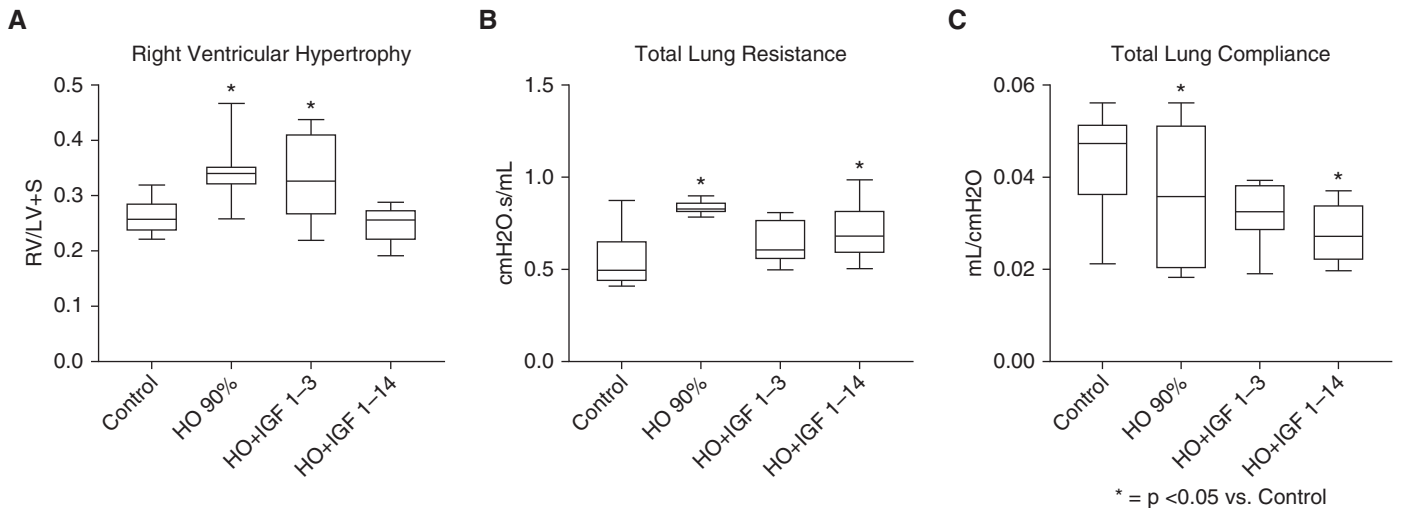
Because angiogenesis is necessary for normal alveolarization (49–51), it has been suggested that protecting the developing pulmonary vasculature from early injury may not only lower PH and improve gas exchange but may enhance distal lung growth and improve long-term respiratory outcomes (42). Clinical studies have shown that early echocardiographic findings of pulmonary vascular disease after preterm birth are strongly associated with the development and severity of BPD and PH at 36 weeks corrected age (52–54). Interestingly, these findings were also associated with a worse respiratory course during the initial hospitalization but also late respiratory outcomes, including respiratory exacerbations, hospitalizations, and the need for asthma medications (53).

Therapeutic strategies that target enhanced endothelial survival, function, and growth may provide novel approaches toward the prevention of BPD after early diagnosis of high risk within preterm populations.

Our findings of beneficial effects of exogenous IGF-1 treatment in preserving lung structure and function in experimental models of BPD further support previous works that have identified a critical role for IGF-1 in mediating normal lung development. IGF-1 regulates fetal growth and organogenesis by stimulating cell proliferation, maturation, and differentiation throughout fetal life and during the early postnatal period (55–62). Lung IGF-I mRNA expression is high during fetal life and decreases after birth (61, 62). Studies using genetic mouse models found that IGF-1 and the IGF receptor-1 play key roles during lung



**Figure 10.** Effects of short (1–3 d) or long (1–14 d) rhIGF-1 (recombinant human insulin-like growth factor-1)/BP3 (binding peptide 3) treatment on distal lung structure in infant rats at 14 days after concurrent exposure to hyperoxia (HO). (A) As shown, hyperoxia decreases lung septation when compared with saline control rats. (A and B) Postnatal treatment with IGF-1 for the entire 14-day exposure but not for 3 days improves lung structure, as reflected by increased RAC (A) and vessel density (B). Micrographs are representative and were obtained at the same magnification (scale bars, 100  $\mu$ m; box plots represent median, interquartile range, and minimum–maximum data points). Animal numbers per study group: control rats = 15; HO = 10; rhIGF-BP3 Days 1 to 3, 2 mg/kg = 10; and rhIGF-BP3 Days 1 to 14 2 mg/kg = 15. HPF = high-powered field; RAC = radial alveolar count.



**Figure 11.** (A–C) Effects of short (1–3 d) or long (1–14 d) rhIGF-1 (recombinant human insulin-like growth factor-1)/BP3 (binding peptide 3) treatment on distal right ventricular function (A) and lung function (B and C) in infant rats at 14 days exposure to hyperoxia (HO). Box plots represent median, interquartile range, and minimum–maximum data points. Animal numbers per study group: control rats = 15; HO = 10; rhIGF-BP3 Days 1 to 3, 2 mg/kg = 10; and rhIGF-BP3 Days 1 to 14, 2 mg/kg = 15. LV+S = left ventricle plus septum; RV = right ventricle.

development *in utero* because fetal lungs in mice with downregulation of IGF-1 or IGF-1R are small and have abnormal interstitial and distal lung structure (60, 61). In addition, impaired growth and angiogenesis has been demonstrated in fetal LECs in an experimental IUGR model due to heat chamber–exposed pregnant ewes (62). We report decreased lung IGF-1 mRNA expression in neonatal lungs of rat pups exposed to intraamniotic ETX, further supporting the concept that early (antenatal) disruption of IGF-1 signaling may contribute to the pathobiology of BPD.

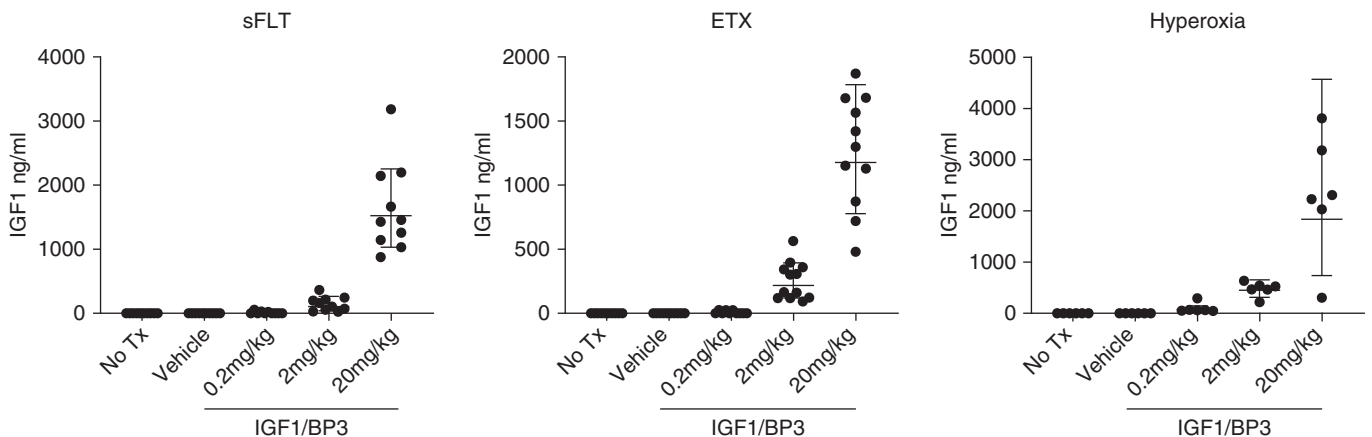
Clinically, circulating IGF-1 levels rise during the third trimester *in utero* but

remain low in neonates who are born premature (38, 63). Importantly, low circulating IGF-1 levels were found in cord blood samples from newborns delivered in pregnancies complicated by PE (63), and low IGF-1 levels have been further associated with the subsequent development of BPD (37, 64). Recently, published data from a multicenter randomized trial of rhIGF-1/BP3 for the prevention of severe retinopathy of prematurity failed to show significant differences as related to the primary retinopathy of prematurity endpoint (65). However, this small pilot trial had the striking finding that preterm infants that received early and prolonged rhIGF-1/BP3 infusions

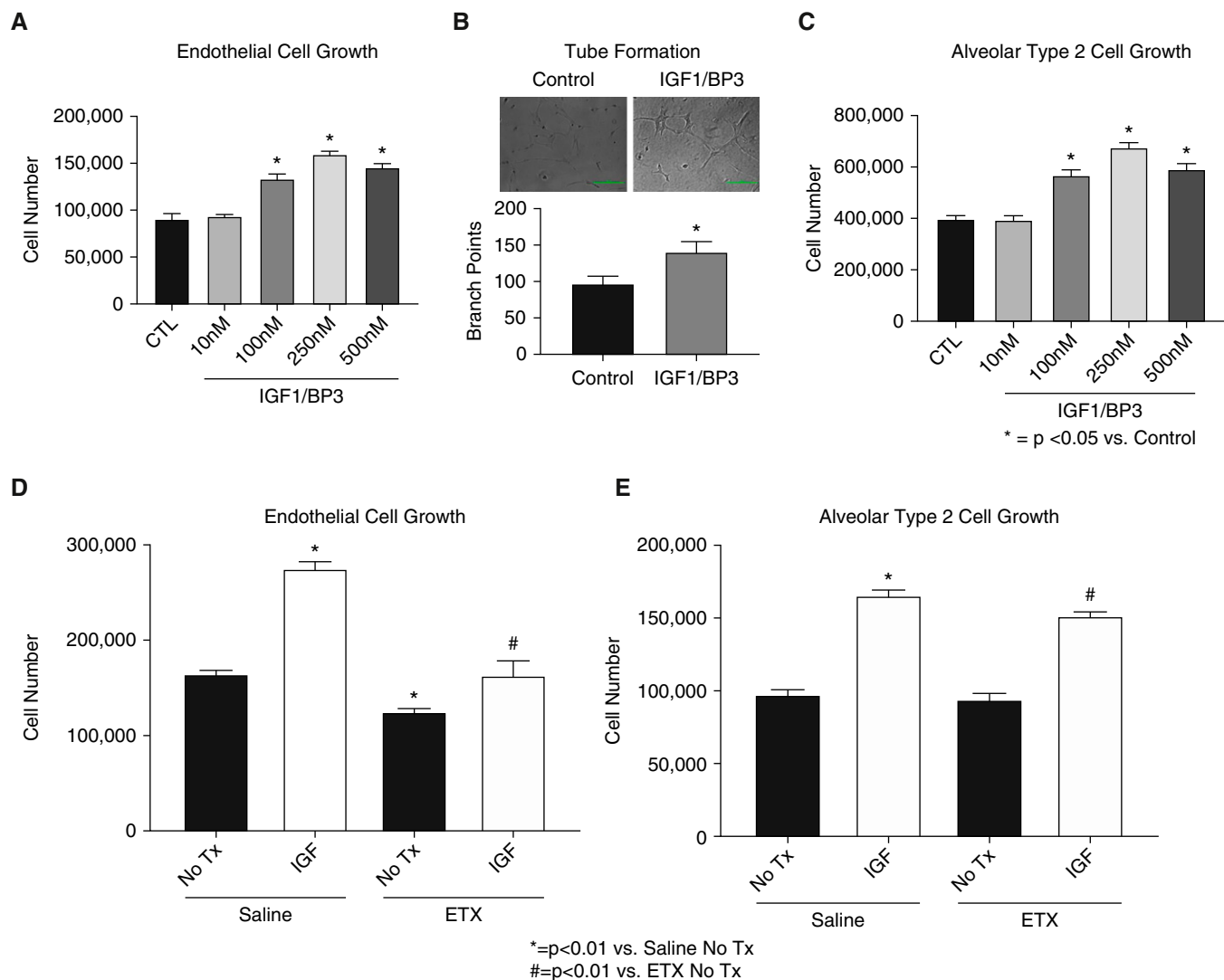
successfully achieved higher circulating IGF-1 levels and had a marked reduction in the risk for severe BPD by 53% in the full analysis set and by 89% in the evaluable set when compared with control rats (65). Although not the primary study endpoint, this striking reduction in BPD severity supports the concept that early restoration of circulating IGF-1 levels may improve respiratory outcomes in preterm infants.

**Conclusions**

We report that rhIGF-1/BP3 treatment promoted lung angiogenesis, preserved lung airspace growth and structure, improved



**Figure 12.** Dose-related changes in serum IGF-1 (insulin-like growth factor-1) levels in rats after rhIGF-1 (recombinant human IGF-1)/BP3 (binding peptide 3) infusion in rats exposed to (left) sFlt-1 (soluble fms-like tyrosine kinase 1), (middle) ETX, or (right) postnatal hyperoxia. Levels were measured from samples drawn at 60 minutes after injections of rhIGF-1/BP3 at Day 14 of life (dose range: 0.2–20 mg/kg). All data points are shown, as well as median and interquartile range. ETX = endotoxin; Tx = therapy.



**Figure 13.** (A and B) Effects of rhIGF-1 (recombinant human insulin-like growth factor-1)/BP3 (binding peptide 3) treatment on neonatal rat pulmonary artery endothelial cell (LEC) growth (A) and tube formation (B) *in vitro*. As shown, rhIGF-1/BP3 enhanced LEC growth and tube formation in a dose-related fashion. (C) IGF-1 treatment also stimulates neonatal rat alveolar type 2 cell growth. (D and E) In comparison with control rats, LECs (D) and alveolar type 2 cells (E) harvested after antenatal ETX exposure had decreased basal growth, which was increased with IGF-1 treatment. Bars represent mean  $\pm$  SEM. CTL = control; ETX = endotoxin; Tx = therapy.

lung function, and prevented the development of RVH in three diverse experimental models of BPD, including two antenatal models that better reflect the new BPD. Overall, these findings support

concepts that early disruption of IGF-1 signaling after premature birth may play an important role in the subsequent development of BPD and PH, and that IGF-1 treatment may provide a novel treatment

strategy for improving cardiorespiratory outcomes in preterm infants. ■

**Author disclosures** are available with the text of this article at [www.atsjournals.org](http://www.atsjournals.org).

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