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Angiopoietin 1 Protects Against LPS-induced Acute Lung Injury and Alveolar Remodeling in Neonatal Mice

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Background

Sepsis-induced acute lung injury (ALI) in premature newborns is a risk factor for bronchopulmonary dysplasia (BPD). Angiopoietins are vascular growth factors that regulate endothelial homeostasis and inflammation. During sepsis-induced ALI, angiopoietin 2 (ANGPT2) is upregulated and antagonizes normal angiopoietin 1 (ANGPT1)-mediated Tie2 receptor phosphorylation resulting in endothelial (EC) immune activation, and subsequent pro-inflammatory signaling. In this study, we investigated the hypothesis that recombinant ANGPT1 will attenuate sepsis-induced ALI and subsequent alveolar remodeling by restoring EC quiescence.

Objectives

- 1) Determine whether recombinant ANGPT1 antagonizes ANGPT2-mediated Tie2 dephosphorylation to suppress LPS-induced ALI.
- 2) Assess the effect of recombinant ANGPT1 therapy on LPS-induced lung remodeling and alveolar simplification.

Methods

6-day-old C57BL/6 mice were given i.p. LPS (2mg/kg) ± 2hr pretreatment with i.p. recombinant mouse ANGPT1 (rmAngpt1, 1µg). Lungs were harvested after 24hrs for mRNA and protein studies, as well as immunohistochemistry. A similar approach was used for bronchoalveolar lavage in 10-day-old mice. For long-term studies on alveolar remodeling, 7-day-old mice were given i.p. LPS ± 2hr pretreatment with i.p. rmANGPT1, and inflation-fixed lung collected on day of life 14 was used for morphometry. Separately, primary fetal HPMEC (ScienCell) cultures were stimulated with recombinant human ANGPT2 (rhANGPT2) or LPS ± recombinant human ANGPT1 (rhANGPT1) for 1 or 24hrs.

Results

rmAngpt1 protected against LPS-induced Tie2 dephosphorylation and ANGPT2 upregulation in mouse lungs and HPMEC. In HPMEC, exposure to rhANGPT2 induced inflammatory mediators and dephosphorylated Tie2, while co-stimulation with rhANGPT1 attenuated both effects. In mice, rmAngpt1 pretreatment acutely suppressed lung NF-κB activation, cytokines, adhesion molecules, and apoptosis. rmAngpt1 also reduced LPS-induced alveolar neutrophils, total cell counts, and protein levels. Early LPS-induced lung tissue destruction was attenuated by rmAngpt1, evidenced by reduced MMP9:TIMP1 levels and elastin fiber degradation. rmAngpt1 also protected against LPS-induced alveolar simplification.

Conclusions

Exogenous ANGPT1 attenuates LPS-induced ALI and alveolar remodeling in newborn mice while antagonizing ANGPT2-induced pro-inflammatory Tie2 dephosphorylation. Restoring balance in Angiopoietin-Tie axis signaling during sepsis with therapeutic ANGPT1 has the potential to counter neonatal sepsis-induced ALI and prevent BPD.

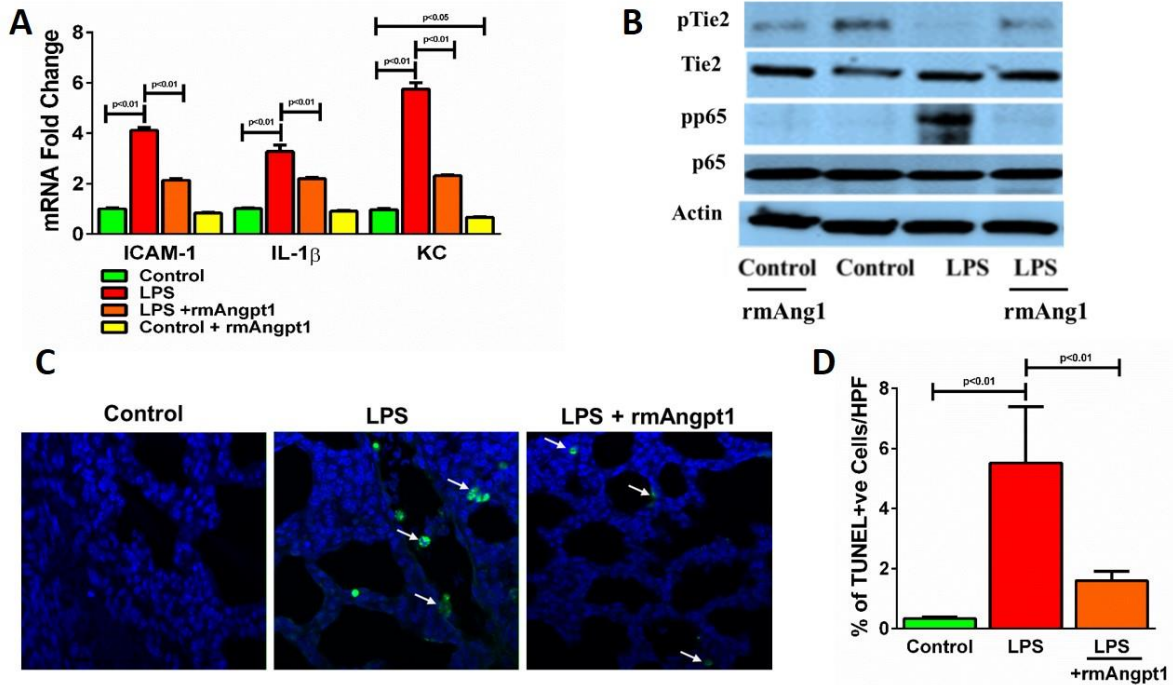


Figure 1- rmAngpt1 pretreatment in 6-day-old mice attenuates LPS-induced lung injury at 24 h (A) PCR done on lung lysates for cytokine gene expression. (B) Western blot on lung lysates showing Tie2 phosphorylation, p65 (NF- κ B) phosphorylation, and ICAM-1. (C) Images depicting TUNEL staining, with quants shown (D).

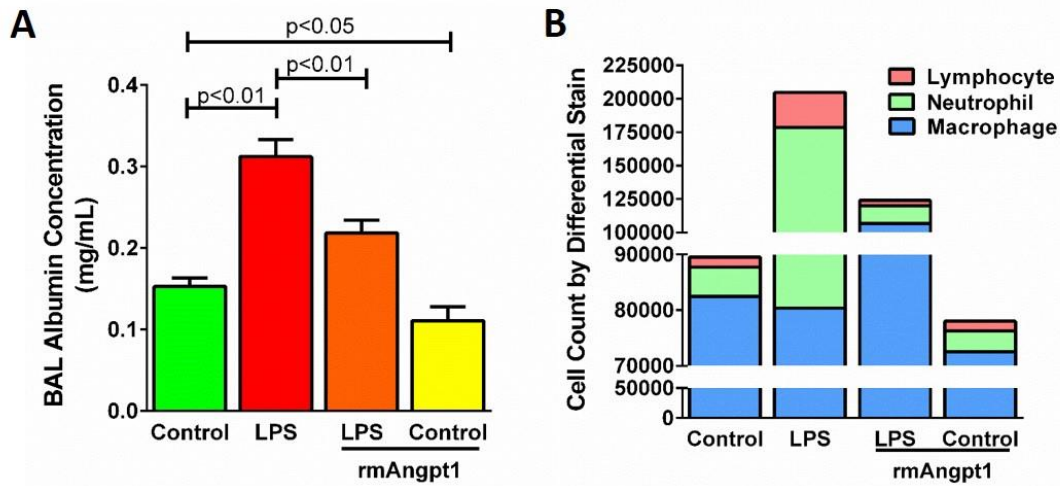


Figure 2- rmAngpt1 pretreatment in 10-day-old mice suppresses LPS-induced alveolar protein content and cell counts. (A) Albumin was quantified in Bronchoalveolar (BAL) fluid after LPS and rmAngpt1 treatments. B) Cell counts were quantified in BAL fluid, and cell differential evaluated by Diff-Quik staining.

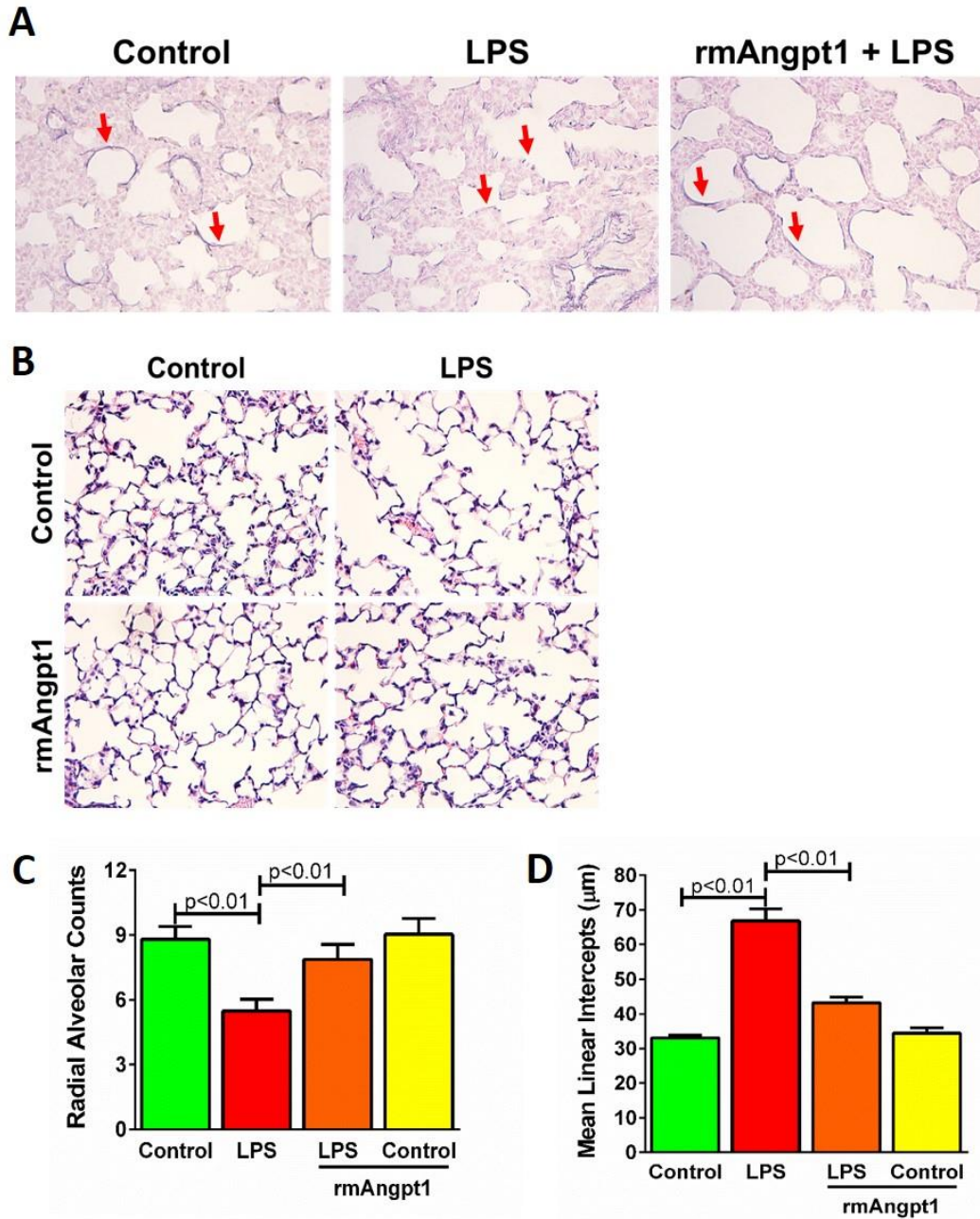


Figure 3- Effect of rmAngpt1 on LPS-induced elastin architecture at 24 h and LPS-induced alveolar remodeling on P14. (A) Elastin fiber staining done in experimental groups 24 h after LPS treatment on P7. B) H&E stained images done on inflation-fixed lungs on P14 after LPS and rmAngpt1 treatments on P7 with RAC (C) and MLI (D) quantified. (C) Graph depicting the radial alveolar counts at P14. (D) Graph depicting mean linear intercepts at P14. Red arrows show elastin fiber staining that is normal (C, LPS+rmAngpt1) or interrupted (LPS).

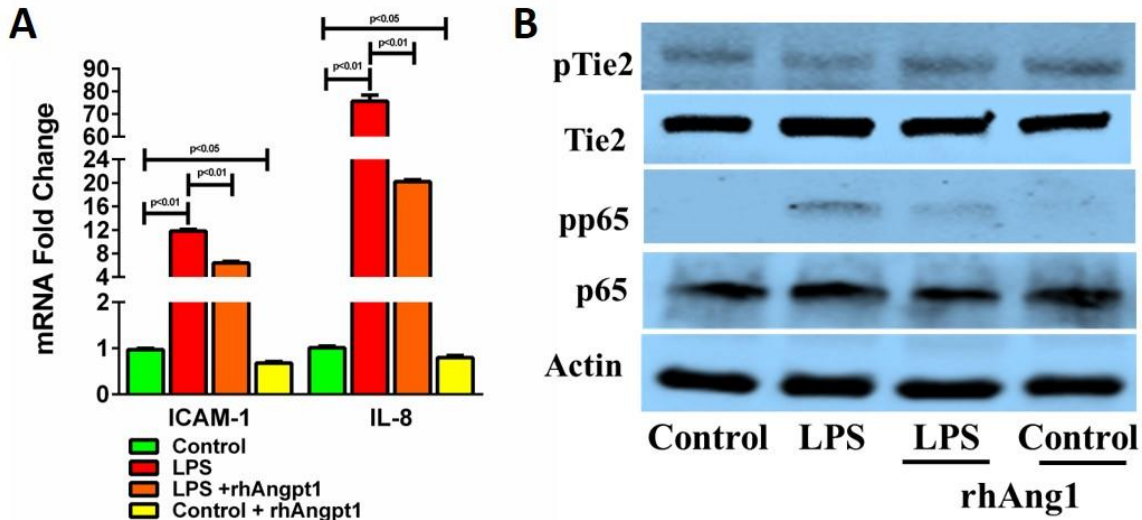


Figure 4- rhAngpt1 suppresses LPS-induced inflammation, NFKB activation, and Tie2 dephosphorylation at 24 h in human pulmonary microvascular endothelial cells (HPMEC) *in vitro*. HPMEC in culture were treated with LPS and rhANGPT1 for experiments. (A) PCR on HPMEC lysates was done for cytokines gene expression after treatments. (B) Western blotting was done in HPMEC lysates for Tie2, phosphor-Tie2, (p)NFKB and NFKB.