Natural anti-infective pulmonary proteins: In vivo cooperative action of surfactant protein SP-A and the lung antimicrobial peptide SP-B^N

Juan Manuel Coya*†, Henry T. Akinbi‡, Alejandra Sáenz*†, Li Yang‡, Timothy E. Weaver‡, and Cristina Casals*†ξ

Supplemental Data

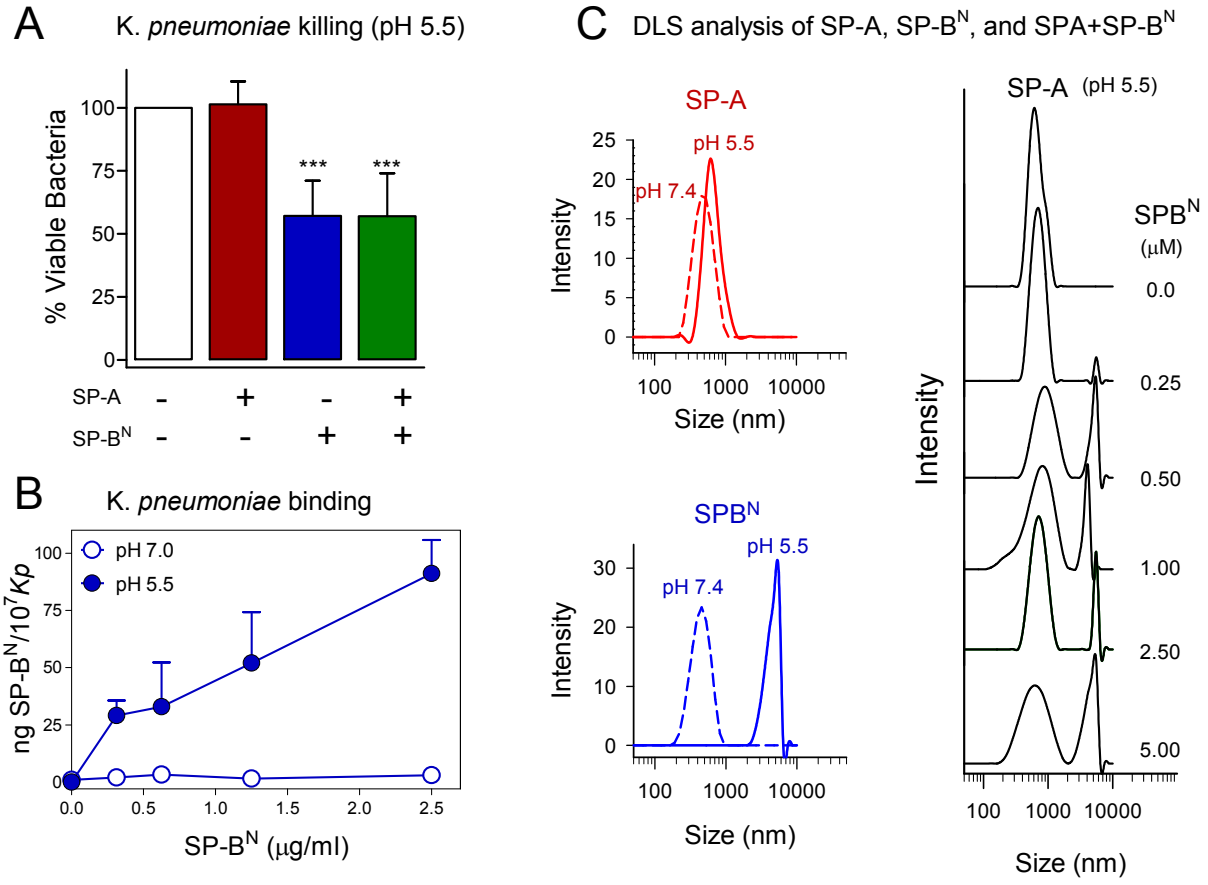

FIGURE S1. A) *K. pneumoniae* K2 killing at acidic pH. 10^5 CFUs/ml of *K. pneumoniae* K2 were incubated for 1h at 37 °C with 10 μg/ml of SP-B^N in the absence or presence of 100 μg/ml SP-A in 5 mM sodium acetate buffer, pH 5.5. Then bacteria were plated on LB agar for CFU count after 18h of incubation at 37 °C. Results are shown as % viable bacteria (percentage of live colony counts compared to untreated control) and are means ± SD of 4 experiments, each duplicated (***$p < 0.001$ *vs*. untreated group). **B) Binding of SP-B^N to K. *pneumoniae* K2 at neutral and acidic pH.** Increasing concentrations of biotinylated SP-B^N were incubated with 10^7 CFUs of *K. pneumoniae* K2 in either 5 mM Tris HCl buffer (pH 7.4) or 5 mM sodium acetate buffer (pH 5.5)

containing 150 mM NaCl at room temperature for 30 minutes. Total *Klebsiella*-associated SP-B^N was measured by solid-phase assay. Results are means ± SD of 4 experiments, each one in triplicate. **C) DSL analysis of the hydrodynamic diameter of SP-A, SP-B^N or SP-A+SP-B^N mixtures at acidic pH**. (*Upper left*) Hydrodynamic diameter of SP-A in either 5 mM Tris HCl buffer (pH 7.4) (460 ± 60 nm) or 5 mM sodium acetate buffer (pH 5.5) (660±140 nm) containing 150 mM NaCl and 175 μM $CaCl_2$. (*Lower left*) Hydrodynamic diameter of SP-B^N at neutral (440 ± 60 nm) and acidic (> 5000 nm) pH; Both Tris HCl and sodium acetate buffer contained 150 mM NaCl and 175 μM $CaCl_2$. The y axis represents the relative intensity of the scattered light; the x axis denotes the hydrodynamic diameter of the particles present in the solution. One representative experiment of 4 is shown. (*Right*) Addition of increasing concentrations of SP-B^N (ranging from 0 to 5 μM) to a solution containing a constant concentration of SP-A (10 nM) in 5 mM sodium acetate buffer, pH 5.5, containing 150 mM NaCl and 175 μM $CaCl_2$ did not change the aggregation characteristics of either SP-A or SP-B^N. One representative experiment of 4 is shown.