[C64] [Poster: A71] Structural Domains of SP-A Required for Induction of Membrane Permeability in Histoplasma Capsulatum

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Rationale and methods: SP-A kills Histoplasma capsulatum (Hc) by inducing membrane permeability. We used site directed mutagenesis to more precisely localize residues of SP-A which are critical for this function. Several mutant proteins were assayed for their ability to increase the access of an alkaline phosphate substrate (ELF97) to the intracellular enzyme, including those with alanine or glycine substitutions for the calcium ligands of the carbohydrate recognition domain (CRD), (E171A, N214A, R197G, E195A), deletion of all domains N-terminal to the neck domain (ΔN1-P80), deletion of the collagen-like domain alone (ΔG8-P80) or disruption of the N-terminal interchain disulfide bond. Data were from n =3, expressed as mean±S.E. and compared by t-test.

Results: The wild type recombinant (wt rec) protein increased membrane permeability by 3.2-fold (p<0.001) over the filtrate control. Excess mannose partially blocked the effect of SP-A on penetration of the ELF97 compared to wt rec control (44.1±6.8 % reduction), as did CRD mutations in calcium binding residues E171A (13.3±3.8% reduction, p>0.05), N214A (33.3±8.5% reduction, p<0.01), R197G (45.0±9.4% reduction, p<0.01), E195A (53.2±0.6% reduction, p<0.01). Deletion of the N-terminal domains (ΔN1-P80) and or the collagen-region alone (ΔG8-P80) reduced the permeabilization activity by 33.7±6.5%, (p<0.01) and 39.6±8.5%, (p<0.01) respectively, but disruption of the major N-terminal interchain disulfide bond had only marginal effect (16.4±28.8% reduction, p>0.05).

Conclusion: We conclude that the Hc permeabilizing activity of SP-A maps to the carbohydrate recognition domain, and that N-terminal domains which contribute to oligomeric assembly are also required for full function.

Tuesday, May 25, 2004 8:15 AM
[**] Thematic Poster Session (Abstract Page: A680) Session: 8:15 am-4:15 pm, SURFACTANT FUNCTIONS
Influence of TNF-α and IL-10 on Uptake of Surfactant like Liposomes into Type II Cells from Injured Lungs

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Rationale: During lung injury surfactant metabolism is affected in type II cells and surfactant secretion from these cells is modulated by TNF-α and IL-10. However there is no information if these cytokines also influence surfactant liposome uptake into type II cells might be of importance for surfactant therapy of injured lungs.

Methods: To study this question type II cells, isolated from rats of controls and from rats exposed to 10 ppm NO2-containing atmospheres for 3 and 20 days, were incubated with surfactant like liposomes in the presence and absence of TNF-α and IL-10 with and without SP-A. Results: In type II cells from all stages of lung injury was clearly elevated. In the cells from the acute lung injury state liposome uptake was elevated by 100% whereas for the chronic lung injury elevation was only about 30%. When SP-A was present in these experiment liposome uptake was doubled in all stages of lung injury. Liposomes that had been internalised in the presence of SP-A remained intracellularly unchanged whereas liposomes internalised without the protein were intracellularly degraded. For cells from normal rats in the presence of TNF-α liposome internalisation was elevated about 20% compared to internalisation without the cytokine. This was also found when the SP-A was present. However there were no marginal differences for the cells from the acute and chronically injured lungs. In contrast, in the presence of IL-10 surfactant like liposome internalisation was reduced by 25% compared to internalisation without the cytokine. This effects was also observed when incubation was performed in the presence of SP-A and also showed no marginal differences in the cells from the acute and chronically injured lungs.

Conclusion: These results indicate that in type II cells from controls and injured lungs TNF-α and IL-10 modulate uptake of surfactant like liposomes.