161 Severe clinical course in cystic fibrosis (CF) patients, compound heterozygous for ΔF508 and haplotype 6

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The ΔF508 deletion is found in 70%, the R553X mutation in 5.3% of CF chromosomes in Switzerland. Both mutations show strong linkage to specific haplotypes generated from the marker allele constellation of XV-2c, KM19, MP6d-9, and J3.11 suggesting that patients carrying the same haplotypes may probably show the same mutation. Age of onset of chronic *Pseudomonas aeruginosa* colonization (AOCP), X-ray scores (Chrispin-Norman), and relative underweight of 35 patients homozygous for ΔF508 (ΔF2), 8 patients compound heterozygous for ΔF508 and R553X (ΔF13) and 13 patients compound heterozygous for ΔF508 and haplotype 6 (ΔF16) were compared. In ΔF2 and ΔF13 patients AOCP begins at the age of 7.0 years (1.3-17.4), whereas in the ΔF16 group the colonisation is already present at the age of 4.3 years (0.4-15.7). The severity of lung disease radiographically determined by the Chrispin-Norman scores is significantly (p=0.03) more progressed in ΔF16 patients (score 7) at the age of 1 year than in the ΔF2 (3.8) and ΔF13 (3.0) groups. Up to the age of 20 years the ΔF16 patients show significant higher scores than the other groups (p=0.04). Due to large standard deviations, underweight did not express significant differences between the three groups. However, the tendencies are the same as for X-ray scores, the ΔF16 patients being most underweighted. Although the clinical course of CF may also be determined by other than genetic factors, we conclude that haplotype 6 predicts a more severe course than do R553X and even ΔF508.

164 ANTIBACTERIAL EFFECT OF PULMONARY SURFACTANT

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The effect of human and of murine pulmonary surfactant (SF) was studied on the outgrowth of Bacillus subtilis, Staphylococcus aureus, Klebsiella pneumoniae, Pseudomonas aeruginosa and Streptococcus pneumoniae. After centrifugation at 300.g, the cell free supernatants of human and murine broncho-alveolar lavage fluids were centrifuged at 25,000.g for collecting SF. The bacteria ($\approx 10^5$) were incubated with SF (2.5 mg/ml) or PBS for 2 hrs at 37°C, washed with PBS and plated overnight. Survival is given as the ratio of the number of colonies after incubation with SF to that of PBS.

Survival	B.subtilis	S.aureus	Kl.pneum	Ps.aerug.	Str.pneum.
human SF:	0.02	0.3	0.8	1.0	n.d.
murine SF:	0.01	0.5	1.0	1.0	1.0

With SF, the outgrowth of the lung pathogens was not inhibited, whereas the proliferation of non-typical lung pathogens was reduced. Proliferation of the sensitive strains was inhibited less at higher numbers of bacteria in inoculum. Conclusion: The antibacterial effect of human and murine surfactant is strongest against non-typical lung pathogens and is inversely related to the number of bacteria. (# Dutch Cancer Foundation: grant 85-84)

PNEUMOLOGY

162 IN VITRO LYMPHOCYTE FUNCTIONS IN THE PRESENCE OF BOVINE SURFACTANT AND ITS PHOSPHOLIPID FRACTIONS

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Endotracheal administration of human or xenogenic surfactant preparations is an effective treatment of respiratory distress syndrome of preterm infants. The application of large amounts of phospholipids to the lung could result in a significant alteration of the local immune response. We thus studied the influence of the bovine surfactant preparation SF-RI 1 (Alveofact®) on lymphocyte functions in vitro.

PHA-induced cell proliferation and immunoglobulin synthesis in the presence of whole surfactant as well as six different defined phospholipids were investgated. A marked concentration-dependent suppression of immunoglobulin production independent of the immunglobulin isotype and cell proliferation was observed in the range of 5 ng/ml - 3 mg/ml of a single phospholipid (or SF-RI 1 respectively). It could be demonstrated that suppression of lymphocyte functions was only due the the phospholipid content of the surfactant preparation.

These data indicate that also in vivo immune functions may be significantly altered by the administration of exogenous surfactant. This may be particulary important in the presence of primary or secondary pulmonary infections.

165 DISTRIBUTION OF EXOGENEOUS SURFACTANT TO THE LUNGS OF RABBITS WITH SEVERE RESPIRATORY FAILURE CAN BE IMPROVED BY REDUCTION OF CONCENTRATION.

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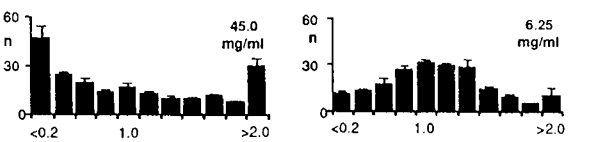
We investigated the initial distribution of endotracheally instilled bovine extracted surfactant (100 mg/kg body weight) to the lungs of rabbits with severe respiratory failure. 141Ce microspheres were mixed with surfactant suspension of 45.0 and 6.25 mg phospholipids per ml. Thirty minutes following instillation the rabbits (n=4) were killed, the lungs removed and cut into 200 pieces (10-50 mg). The distribution histiograms of the radioactivity per mg in each lung piece are shown in the figure.



We conclude that surfactant treatment administered in a concentration of 6.25 mg/ml results in a more homogeneous distribution than 45.0 mg/ml. This observation may have important clinical impact on surfactant treatment.

163 GRP-LIKE IMMUNOREACTIVITY IN BRONCHIAL SECRETIONS OF SURFACTANT-TREATED PREMATURE INFANTS WITH SEVERE RESPIRATORY DISTRESS SYNDROME (RDS)

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Gastrin-releasing peptide (GRP) and GRP-related peptides are putative growth factors. Existence of GRP-like immunoreactivity (IR) has not been examined in bronchioalveolar secretions of the neonate. We have analyzed bronchial fluid of 54 premature infants (26-33 weeks of gest.) with severe RDS (FiO2 > 0.8, mechanical ventilation). Sequential samples (n = 290) were obtained within 1 week after surfactant (S) replacement therapy (single or multiple doses; total amount of phospholipids 200 vs. 400 mg/kg bw). GRP-like IR was determined by radioimmunoassay. Results: In 32/54 patients (59%) GRP-like IR could be detected. Concentrations were 0,3-70 ng/mg albumin (a). Neither gestational age, birthweight, sex nor severity of pulmonary disease did correlate with the amount of GRP-like IR detected. In 13/24 samples (54%) of single-dose-S-treated infants GRP-like IR was found (x = 3,16 ng/mg a); in multiple treated infants 18/30 probes (60%) were GRP-positive (x = 4,47 ng/mg a). Further HPLC characterization of the GRP-like IR suggests the existence of different molecular forms of GRP-like peptides. Conclusions: GRP-like IR can be detected in bronchial fluid of premature infants with severe RDS. Multiple doses of surfactant do not influence the GRP content. The physiological role of GRP-like peptides in the developing respiratory tract of the neonate has not been defined yet.

166 DEVELOPMENTAL CHANGES IN THE RESPONSE OF TYPE II CELLS TO PHOSPHATIDYLCHOLINE (PC) SECRETAGOGUES.

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PC secretion in adult rat type II cells can be stimulated by several agents acting via different signal-transduction mechanisms. To determine if these mechanisms also operate in developing lung we have compared the response of type II cells isolated from 21 day fetal and 1, 7 and 14 day old rats with those from adults to 36 uM terbutaline, 10 uM N-ethylcarboxamidoadenosine (NECA, adenosine analog), 1 mM ATP, 10 uM tetradecanoylphorbol acetate (TPA) and 25 nM ionomycin. Fetal cells were isolated by trypsin/collagenase digestion and differential adhesion and those from newborns and adults by elastase digestion and panning on IgG-coated dishes. The cells were cultured for 18-20 h with ^{3}H-choline, washed in fresh medium and incubated ± agonists for 90 min after which ^{3}H-PC in cells and medium was measured. The cells were 81-97% type II cells. The rate of basal secretion (PC in medium as % of total in cells + medium) was the same in all 5 groups.

	% secretion	% stimulation (means ± SE; n=2-7 except adult n=4-37)				
	Control	Terbutaline	NECA	ATP	TPA	Ionomycin
Fetal	0.91 ± 0.10	18 ± 7	19 ± 6	30 ± 3	85 ± 17	47 ± 9
+ 1	1.01 ± 0.27	52 ± 9	64 ± 4	74 ± 6	329 ± 29	53 ± 9
+ 7	0.90 ± 0.13	97 ± 19	76 ± 19	156 ± 36	336 ± 53	46 ± 7
+14	0.91 ± 0.07	77 ± 22	90 ± 16	181 ± 43	356 ± 63	42 ± 25
Adult	1.12 ± 0.05	94 ± 7	106 ± 13	301 ± 20	396 ± 24	46 ± 15

However, there was a developmental change in the response to the secretagogues. These data show that different signal-transduction pathways mature at different developmental stages in type II cells. Supported by NIH (HL-31175) and the DFG.