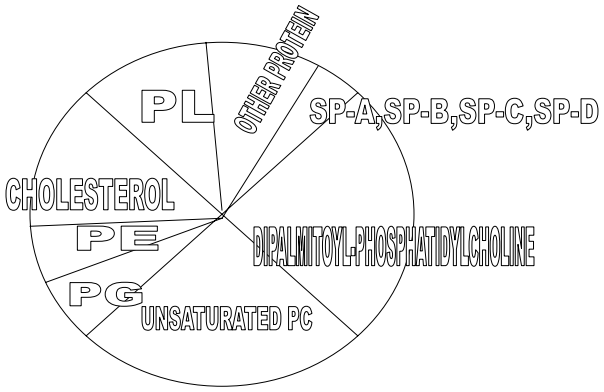
SURFACTANT IN HEALTH AND DISEASE

DR. AMIT RAODEO

INTRODUCTION

- Lung epithelium is in close contact with environment-acts as barrier.
- Appropriate balance between pro and anti inflammatory response is critical.
- Airspace lined with a lipoprotein complex called surfactant – named so because of its ability to reduce surface tension .
- Also plays important role in host defense mechanism.
- SP-A and SP-D,members of collectin family-involved in innate immunity.

COMPOSITION AND SYNTHESIS

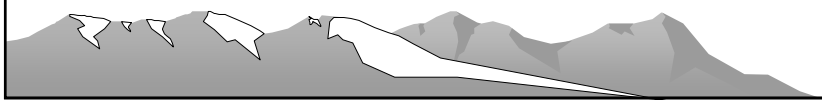


COMPOSITION AND SYNTHESIS

- All components synthesised by Type II alveolar cells.
- Surfactant complex released from intracellular storage granule, the lamellar body into liquid hypophase that covers alveolar epithelium.
- Lamellar body is then transformed into lattice like structure called tubular myelin.
- Monomolecular film which reduces surface tension is formed at air liquid interface.
- Composition of this film is mostly of Dipalmitoylphosphatidylcholine(DPPC).

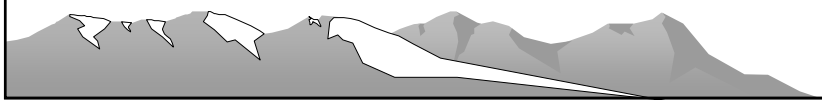
METABOLISM

- Major pathway –
- 1)uptake by TYPE II cells where both recycling and degradation occurs.
  2) significant fraction is degraded by alveolar macrophages.
- Minor pathway-movement up the airway and across epithelial- endothelial barrier.



Surfactant Proteins

- These are SP-A,SP-B,SP-C and SP-D.
- All except SP-C are synthesised by clara cells of airway.
- SP-B and SP-C are highly hydrophobic and facilitate adsorption of lipids to an air- liquid interface.
- SP-A and SP-D are relative hydrophilic and does not contribute to surface reducing property.
- SP-D binds to phosphatidylinositol which is minor component in surfactant isolates.
- The levels are elevated and ratio with phosphatidylglycerol is reversed in disease states like PAP,IPF,ARDS.

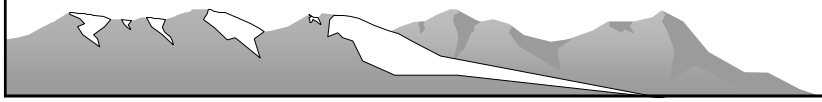


CELLS INVOLVED IN PULMONARY HOST DEFENSE

A) Alveolar Macrophages:

- Most abundant component of BAL fluid (85%).
- Primary defender of alveolus from infection.

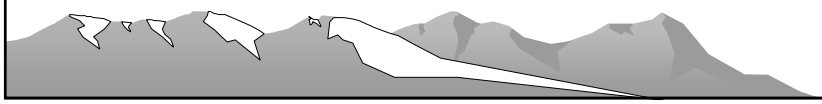
- Role was recognised in 1967 by Green and co-workers.
- Surfactant proteins have been shown to regulate variety of macrophage function.



CELLS INVOLVED IN PULMONARY HOST DEFENSE (CONTD)

B) PMN leucocytes:

- Major cells implicated in variety of noninfectious diseases including IPF, asthma and emphysema and variety of infectious disorder.
- Comprise of 1-3% of cells in BAL fluid.
- Also play important role in ARDS.
- Surfactant inactivation secondary to influx of serum protein is important manifestation of disease leading to increased surface tension and reduced compliance.



CELLS INVOLVED IN PULMONARY HOST DEFENSE (CONTD)

D) Alveolar type II cells:

- Not a traditional immune cell but studies have shown that it may have immunoregulatory function.
- In addition to surfactant, complement components like C2,C3,C4,C5 and factor B are secreted by these cells.
- Other immunoregulatory molecules like IL3,TGF and chemotactic proteins like RANTES.
- Also secrete GM-CSF.
- In addition to activation of alveolar macrophages,it plays important role in regulation of surfactant metabolism.

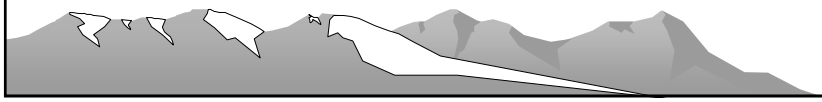
COLLECTINS

- Family of proteins that are nonmembrane bound & involved in innate immunity.
- Plays important role in host defense.
  Consists of :
  1)SP-A and SP-D
  2)MBP (mannose-binding protein)
  3)Conglutinin
  4) CL-43
  5)C1q

SURFACTANT PROTEIN-A

- most abundant surfactant protein.
- Immunocytochemistry studies show that it is concentrated in tubular myelin.
- Method of isolation:

  1)Butanol extraction-most common.
  2)Isoelectric focusing.
  3)Ion exchange chromatography.



SURFACTANT PROTEIN-A (CONTD)

- Functions-

  1)Enhances immunological function of alveolar macrophages.
  2)Stimulate oxidative burst and phagocytosis in these cells.
  3)Interacts with various organisms and enhances their uptake by alveolar macrophages,for example Staph.aureus,Strepto. Pnuemonia,H. influenzae, Cryptococcus,P.carini,Influenza A virus.

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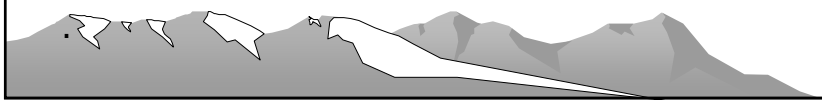
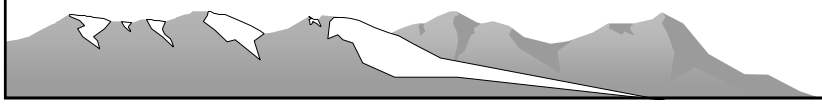


SURFACTANT PROTEIN-A (CONTD)

4)Interacts with LPS of GM –ve organisms.

5)Inhibits superoxide production by alveolar macrophages and prevents free radical injury-immunoregulatory function.

6)Inhibits certain cytokines like IL1,TNF.



SURFACTANT PROTEIN-A (CONTD)

- SP-A levels are significantly elevated in AIDS pts with P.carinii pneumonia as compared to pts without PCP.
- SP-A levels in HIV+ve pts were significantly lower than in healthy volunteers.
- Significant cor-relation between abundance of P.carinii and SP-A lavels.
- This finding is indicator of immunomodulatory function of SP-A.

Stemberg et al;Jour labClin.Med;125;1995

SURFACTANT PROTEIN -D

- It is distributed at limiting membrane.

Functions:

- It binds to Gm-ve bacteria like E.coli,Klebsiella and Pseudomonas.
- It also causes aggregation of influenza virus.
- SP-D along with other collectin including SP-A,MBP and CL-43 are pathogenic to cryptococcus neoformans.
- It enhances superoxide production.
- It is potent chemoattractant.

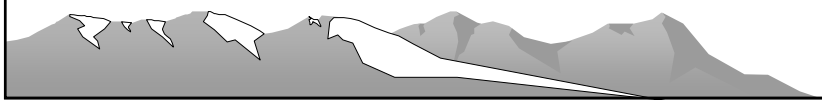
SUMMARY OF IMMUNE FUNCTIONS OF SURFACTANT

Effects of surfactant proteins

- Enhance chemotaxis.
- Enhance phagocytosis.
- Aggregate microbes.
- Enhance killing of some bacteria.
- Alter production of free radicals and cytokines.
- Stimulate immunoglobulin production.
- Alter lymphocyte proliferation.

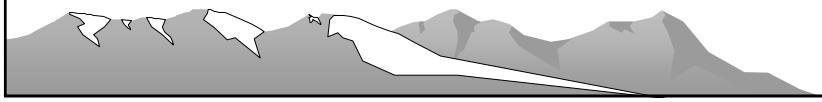
Effects of surfactant lipids

- Enhance phagocytosis and killing.
- Alter free radical production.
- Alter lymphocyte proliferation.



PULMONARY ALVEOLAR PROTIENOSIS

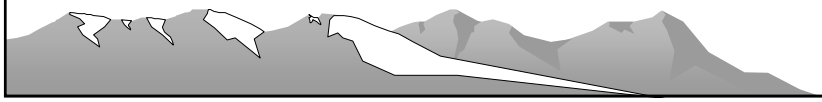
- First described in 1958 by Rosen et. al as a unique disorder characterised by intra-alveolar accumulation of fine granular eosinophilic and PAS+ve material.
- Heterogenous group of congenital, idiopathic and secondary diseases in newborn, infants and adults.
- Idiophathic PAP most common form.
- Characteristic antibody to anti GM-CSF.
- GM-CSF shows therapeutic activity in idiopathic PAP.
- Secondary PAP- rare pulmonary complication after exposure to silica and titanium or in pts with hematological malignancies, lymphomas,acute and chronic leukemias.



PATHOGENESIS

A. Initially thought to be because of impairment of surfactant clearance by alveolar macrophages as a result of inhibition of the action of GM-CSF. Immunohistochemical stains – SP-A,SP-B and SP-D are increased in BAL fluid of PAP.

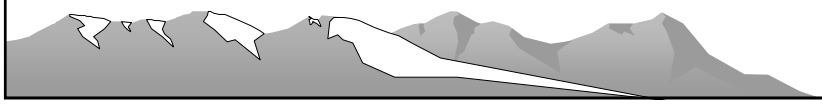
B. Recently with addition of western blotting,significant intra-alveolar accumulation of precursors of SP-B,which is not found in normal lungs is seen.This cannot be explained on the basis of defective clearance.



PATHOGENESIS (CONTD)

- SP-B is synthesised as a pro- protein in Type II pneumocytes.
- Post translational processing occurs between Golgi and mvb prior to transfer to lamellar bodies and secretion in alveolar space.
- Processing intermediate of pro SP-B accumulates in alveolar space in PAP,by passing lamellar bodies due to abnormal secretion of transport vesicles.
- Although SP-C is also secreted by same pathway,precursor of SP-C was uncommonly detected-this occurs due to incomplete pamitoylation of SP-C leading to di- and oligomeric SP-C.

Euro.Resp J 2004;24;426-435



ACUTE RESPIRATORY DISTRESS SYNDROME

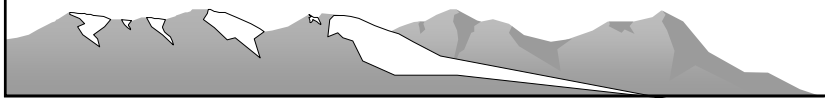
- Clinical syndrome characterised by rapid onset of dyspnoea,hypoxemia and diffuse pulmonary infiltrates leading to respiratory failure.
- Pathophysiology- neutrophil infiltration followed by release of cytokines like IL1,IL8,TNF and LTB4.
- These cytokines damage capillary endothelial cells and type II pneumocytes leading to loss of alveolar capillary barrier which leads to edema formation.
- In addition, there are plasma protein aggregates and dysfunctional surfactant protein accumulation which leads to formation of hyaline membrane.

ACUTE RESPIRATORY DISTRESS SYNDROME

Normal surfactant functions

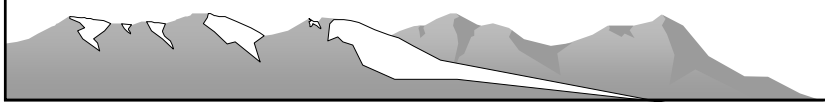
- Reduces alveolar surface tension ----prevents atelectasis.
- Maintains patency of small airways.
- Prevents edema.
- Contributes to host defense against microorganisms. Lack of surfactant function in ARDS contributes to atelectasis,shunt and gas exchange abnormality and predisposes patient to infection and injury from mechanical ventilation.

Thus surfactant has theoretical benefit in treatment of ARDS.



ACUTE RESPIRATORY DISTRESS SYNDROME

- Recombinant surfactant containing surfactant proteins found useful in preclinical studies Roger et. al. did two independent, multicentre,parallel group,double–blind, controlled prospective studies.
- Only difference in them were timing of initiation of therapies-<48 hrs in one group and <72 hrs in other group after the diagnosis.
- Dose-1 mg of recombinant SP-C and 50 mg of phospholipids through ET tube.
- Sample size-110 pts per group.



ACUTE RESPIRATORY DISTRESS SYNDROME

- Conclusion:
  No significant difference in 28 days mortality was found.But pts with surfactant therapy had greater improvement in gas exchange during 24 hr treatment period suggesting potential benefit of longer treatment course.

NEJM 351 ; 9 ; 884-892

Table 1. Baseline Demographic and Physiological Characteristics.*

Characteristic	North American Study		European and South African Study		Pooled Data	
	Control Group (N=115)	Surfactant Group (N=106)	Control Group (N=109)	Surfactant Group (N=118)	Control Group (N=224)	Surfactant Group (N=224)
Sex — no. (%)						
Male	74 (64)	65 (61)	79 (72)	80 (68)	153 (68)	145 (65)
Female	41 (36)	41 (39)	30 (28)	38 (32)	71 (32)	79 (35)
Age — yr	53.1±17.6	56.5±17.8	53.0±18.0	50.6±17.5	53.0±17.8	53.4±17.9
APACHE II score†						
Median	17	19	17	16.5	17	18
Mean	17.9±6.6	18.6±6.1	16.6±5.8	17.4±7.5	17.2±6.3	18.0±6.9
<11 — %	9	12	17	21	13	17
11–23 — %	79	65	72	60	75	62
>23 — %	12	23	11	19	12	21‡
SOFA score§	7.2±3.3	8.1±3.6	8.7±3.5	8.6±3.5	7.9±3.5	8.4±3.6
Tidal volume — ml/kg of body wt	7.2±2.1	7.5±2.3	7.7±2.1	7.7±2.2	7.4±2.1	7.6±2.2
P_{plat} — cm of water	29.5±6.9	29.9±7.9	24.4±7.1	26.0±7.8	27.4±7.4	28.2±8.5
PEEP — cm of water	10.6±3.6	11.2±3.5	10.7±3.1	11.1±3.1	10.7±3.4	11.1±3.3
FiO_2 — mm Hg	0.65±0.16	0.65±0.17	0.71±0.16	0.70±0.17	0.68±0.16	0.68±0.17
PaO_2:FiO_2	130±39	132±40	136±39	137±40	133±39	136±41

* Plus–minus values are means ±SD. P_{plat} denotes end-inspiratory plateau pressure, PEEP positive end-expiratory pressure, FiO_2 fraction of oxygen in inspired gas, and PaO_2 partial pressure of oxygen in arterial blood.
† Scores on the Acute Physiology and Chronic Health Evaluation (APACHE II) can range from 0 to 71, with higher scores indicating more severe illness.
‡ P=0.007 for the comparison with pooled data for the control group.
§ Sequential Organ-Failure Assessment (SOFA) scores range from 0 to 4 for each organ system, with higher aggregate scores indicating more severe organ dysfunction. We defined failing organs as those with an individual score of 3 or 4.

Table 3. **Outcome Measures.**

Outcome	North American Study		European and South African Study		Pooled Data	
	Control Group (N=115)	Surfactant Group (N=106)	Control Group (N=109)	Surfactant Group (N=118)	Control Group (N=224)	Surfactant Group (N=224)
Ventilator-free days						
Median	6.0	3.5	0.0	0.0	1.0	0.0
68% range	0.0–21.0	0.0–21.0	0.0–20.0	0.0–19.0	0.0–20.0	0.0–20.0
Area under PaO_2:FiO_2 time curve from baseline to 24 hr (mm Hg•hr)*	417±984	809±1397†	319±802	554±1052‡	369±900	674±1229§
Alive at day 28 (%)	75	68	61	61	68	64

* The values are empirical means ±SD.
† P=0.02 for the comparison with the control group by the t-test in a univariate analysis.
‡ P=0.06 for the comparison with the control group by the t-test in a univariate analysis.
§ P=0.003 for the comparison with the control group by the t-test in a univariate analysis.

ACUTE RESPIRATORY DISTRESS SYNDROME

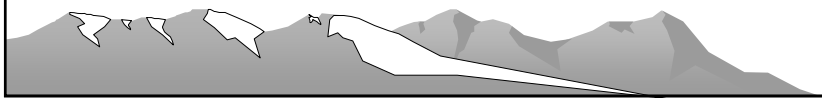
- Post hoc analysis of direct vs indirect lung injury causing ARDS.
- The interaction was significant for mortality analysis(P=0.002) but did not reach significance for analysis of number of ventilator free days.
- This indicates that among pt. with direct ARDS,those who received surfactant tended to have higher survival than those receiving standard therapy.

CHRONIC OBSTRUCTIVE PULMONARY DISEASE

- SP-A levels were studied in 30 pts of chronic bronchitis.
- Sr. cotinine and nicotine levels were measured as a surrogate marker of smoking.
- SP-A levels were compared with Sr. cotinine and nicotine levels in smokers and non-smokers.
- Results showed that significant rise in levels of SP-A in smokers ($P<0.05$) as compared with non-smokers.

  Further studies required to find out whether SP-A can be used as a marker for early identification of smokers at risk for COPD.

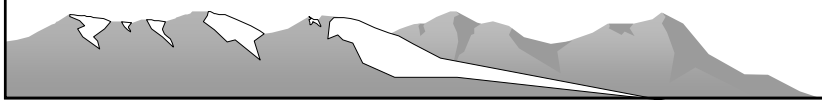
D.Behera et. al. Indian J.Chest Dis Allied Sci 2005;47



Correlation of SP-A & SP-B levels with smoking

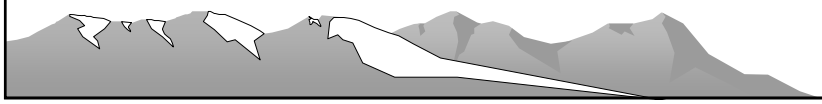
- Presence of SP-A ,SP-B & CC16 (Clara cell associated protein) in serum is termed as pneumoproteinaemia.
- Robin et al in their study found that SP-A and SP-B levels are markers of smoking in healthy individuals.
- Smoking related increase in alveolo-capillary leakage resulted in increased SP-B levels.
- Toxic effect on clara cells result in decreased CC16 levels.

Eur.Resp. J. Nov.2002



INTERSTITIAL PULMONARY FIBROSIS

- Mortality 50% in 5 yrs.
- Current therapies are marginally effective in improving survival and lung function.
- SP-A and SP-D are significantly elevated in pts with IPF and systemic sclerosis where as in same study they found that this does not correlate well in cases of sarcoidosis, beryllium disease and normal controls.
- SP-D levels correlate with radiological abnormality.
- SP-A and SP-D levels are highly predictive of survival.



INTERSITIAL PULMONARY FIBROSIS (CONTD)

- Multivariate analysis was done comparing serum SP-A and SP-D to other commonly measured predictors of survival in IPF.
- Used largest North American data set.
- 142 pts of IPF vs healthy volunteers were involved.
- SP-A and SP-D were compared with CRP score (Clinico Radiographic Physiologic) which includes
  1)Dyspnea index
  2)CXR
  3)PFT
  4)Formal exercise study

Greene et. al. ; Euro Resp J 2002 ;19 ; 439-446

HIGH ALTITUDE PULMONARY EDEMA

- Mechanism- Increase in capillary permeability due to
  1) Inflammatory reaction
  2)Free radical mediated injury.
- SP-A, potent anti-inflammatory and most abundant of surfactant proteins protects growing cells from oxidative injury.
- SNP (single nucleotide polymorphism) of SP-A1 and SP-A2 genes is associated with
  1)ARDS
  2)COPD
  3)PULMONARY INFECTIONS

HIGH ALTITUDE PULMONARY EDEMA

Study conducted by institute of genomics and integrative biology (Mr.Saxena,Dr. Madan and Sharma),New Delhi.

- Genetic polymorphism in SP-A1 and SP-A2 is associated with increased incidence of HAPE.

They studied 46 pts- out of which

- 12 pts were low altitude natives with HAPE.
- 15 pts were low altitude natives and healthy.
- 19 pts were high altitude natives and healthy

Chest 2005 Sept 128(3);1671-1679

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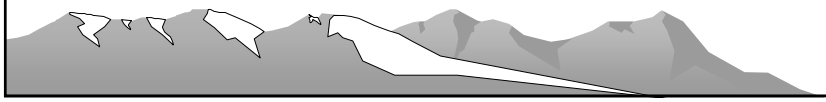
EXTRINSIC ALLERGIC ALVEOLITIS

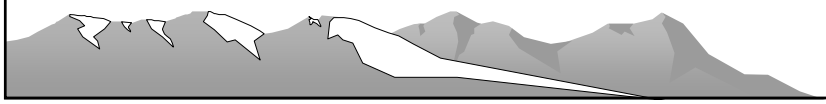
- Pathophysiology involves oxidative lung damage as well as interstitial & alveolar inflammation.
- Macrophages & mast cells are the predominant cells involved.
- Produce LTs & PGD2.
- SP-D & Urinary eicosanoid metabolite 8 iso PGF2 correlate with disease activity.
- Levels are markedly elevated in acute exacerberation.
- Steroids suppress their levels.

Hagashi et al , Eur. Resp. J. 2005;26;1069-73



CONCLUSION

- Surfactant plays important role in normal lung function.major role in reducing surface tension and preventing collapse of alveoli.
- It also plays important role in host defence against various infections,inflammatory conditions and allergies.
- Serum levels of surfactant can be used as biomarker in certain diseases like extinsic allergic alveolitis,IPF,etc.
- Surfactant has therapeutic role in certain diseases like RDS of newborn.
- Recent studies:Potential role of Recombinant forms of SP-A and SP-D in controlling pulmonary infections,inflammation and allergies in humans.




THANK YOU