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Airway inflammation is reduced in workers exposed to protein from bacteria one year after cessation of exposure

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1

Norferm – bacterial single cell protein production plant

2

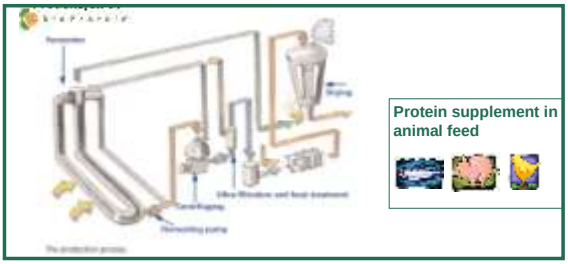
Fish feed production from bacteria

Production plant:

- Norferm – A plant localized on the west coast of Norway close to Norwegian oil prouction (Statoil)
- Production of protein supplement for animal feed is based on fermentation on methan gas as the main source for energy
- Production started 1998
- Production plant closed down in 2006

3

Production



4

Background

- New technology
- Employees reported occasional episodes of:
  - Fever / flue like symptoms
  - Fatigue
  - Sore and irritated eyes
  - Dry skin and hand eczema
- Generally in good health and low frequency of sick leave

5

Content of the fish feed

Bioprotein ®

- Baceria used in the production:
  - 90 % *Methylococcus capsulatus*
  - 10 % *Alcalligenes*
  - *Bacillus species*

Methyl

- **Contain whole bactria and all components present in living bacteria**
  - **14 000 Endotoxin unit (EU)/mg**

- Final product
  - 70% protein, 12 % carbohydrates, 10 % fat, 7 % minerales, 1 % fiber

6

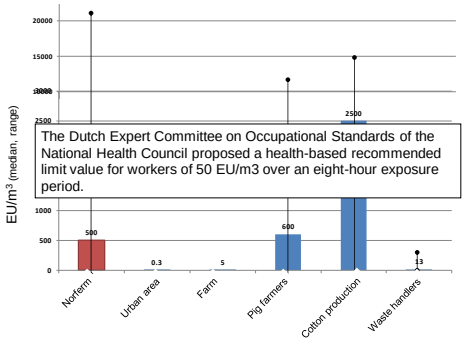
Endotoxin

- Fragments of the cell wall of gram negative bacteria. Contain lipopolysaccarides (LPS), proteins and lipids
- LPS: glycolipid causes a general pro-inflammatory response induced by infection/inflammation

Is LPS causing the health problems?

7

Endotoxin exposure



8

Hypotheses
One year after cessation of exposure:

- Lung function is improved?
- Systemic inflammatory markers are normalized?
- Airway inflammation is reversible?

9

One year after cessation of exposure.

- Lung function changed
  - In the low exposure group, but not in the high exposure group, there were significant improvements in both forced vital capacity (FVC) (290 ml) and forced expiratory volume in 1 s (FEV1) (180–210 ml) (p=0.004–0.03)
- Systemic inflammation markers decreased
  - The number of leukocytes and eosinophilic cationic protein and D-dimer levels increased significantly with increasing endotoxin exposures and decreased significantly 1 year after exposure termination.

Skogstad M et al. Occup Environ Med 2012;69:107-112

Subjects and Methods

- 24 non-smokers (4 ex-smokers – quitted smoking at least one year before the first sputum examination)
- Induced sputum (longitudinal design)
  - 2004/2005 (exposed period)
  - 2007 (one year after closure of the plant)

11

Induced sputum

- Spirometry
  - Before start of first examination, and between inhalations
- Inhalation of hypertone saline for 7 minutes (3%, 4% og 5%)
- Coughing and sputum sampling between each inhalation
- Important:
  - Enough cells
  - Minimal contamination of cells from upper airways
  - Viability (keep sample on ice)
- Celles harvest from central airways

12

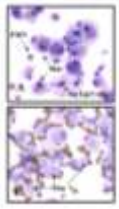
Field work – 500 km from the University



13

Analysis of sputum

- Cells
  - Total number of cells
  - Differential cell counting
- Supernatant
  - Protein analyses
- Celleisolering
  - Gene expression
- Flow cytometry
  - Cell quantity and quality
  - Celle activity



14

Inflammation markers induced by Bioprotein

- Complement
  - Classical pathway: C1-inh/C1rs
  - Classical and Lectin pathway : C4bc
  - Alternative pathway : C3bBbP
  - TCC: Terminal Complement Complex
- Proinflammatory cytokines:
  - IL-8 (CXCL8), MCP-1 (CCL2), MIP-1α (CCL3), MIP-1β (CCL4)
- Chemokines:
  - IL-1β, IL-6, TNFα og IFNγ
- Cytokines and Growth factors:
  - IL-1RA, IL-4, IL-9, IL-17, G-CSF, VEGF

15

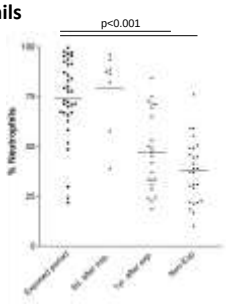
Characteristics

	*Exposed period (n=24)	†One year after exposure (n=24)
Age	37 (7)	40 (7)
Gender (M/F)	18 , 6	18 , 6
Yrs employed	5.9 (2.1)	
FEV1% pred.	86 (31)	97 (7)
FVC% pred.	90 (7)	102 (11)
FEV1/FVC%	80 (7)	78 (6)
B-leucocytes	6.4 (2.7)	6.1 (1.4)
CRP	1.8 (2.1)	2.3 (4.3)

FEV1= Forced expirtory flow 1.0 sec, FVC=Forced vital capacity. Data presenteed as Mean(SD), Data collection *2004-2005, †2007

16

Neutrophils

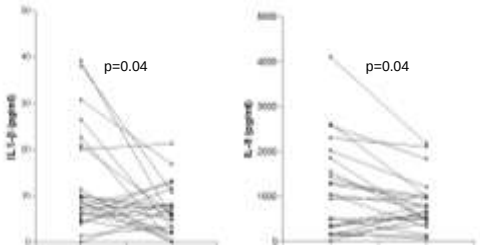


-eosinophils very low values

(Sikkeland LIB et al. Inhal Toxicol. 2009 Jul;21(8):674-81)

17

Inflammation markers in sputum supernatant

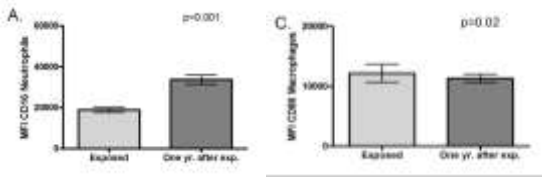


18

– LPS has *in vitro and in vivo studies been shown to* change phenotypes of the cell surface. Is this the case also among BSCP production workers?
  • Exposed sample compared with unexposed sample one year after cessation of exposure

19

Surface markers (flow cyt. analyses)



Conclusions of flow cyt. results:

Immune reaction showed a shift towards antibody mediated response during BSCP exposure.

20

(Sikkeland LIB et al. Recovery From Workplace-Induced Airway Inflammation One Year After Cessation of Exposure, submitted)

Conclusions

- Workers exposed to bacterial single cell production in the fish feed industry have
  – General airway inflammation
    • Neutrophilia
    • No eosinophilia (less likely allergic induced inflammation)
- During exposed periode
  • Cytokine profile as seen for a general immune response
  • Simultaneously trigging of the spesific (antibody mediated) immune defence (↑CD86, ↓CD11b, ↓CD16)
- Inflammation reversible one year after cessation of exposure

21

Some unsolved questions

- May continuous and long-lasting exposure of BSCP induce chronic structural changes of the airways like COPD (neutrophil inflammation)?
- Consequences?
  – Primary prevention (reduction of exposure)
  – Important to surveil workers in this type of industry with regular measurements of respiratory questionnaires and spirometry to detect chronic symptoms or increased decline in lung function

22