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

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Fish feed production from bacteria

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

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

Production

Content of the fish feed

Bioprotein®
- Bacteria used in the production:
  - 90% Methyllococcus capsulatus
  - 10% Alcaligenes
  - Bacillus species
- Contain whole bacteria and all components present in living bacteria
  - 14 000 Endotoxin unit (EU)/mg
- Final product
  - 70% protein, 12% carbohydrates, 10% fat, 7% minerals, 1% fiber

Norferm – bacterial single cell protein production plant
Endotoxin

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

Is LPS causing the health problems?

Hypotheses
One year after cessation of exposure:

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

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.


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)

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
Field work – 500 km from the University

Analysis of sputum

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

Inflammation markers induced by Bioprotein

- Complement
  - Classical pathway: C3-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

Characteristics

<table>
<thead>
<tr>
<th></th>
<th>Exposed period (n=24)</th>
<th>1 year after exposure (n=24)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Age</td>
<td>37 (7)</td>
<td>48 (7)</td>
</tr>
<tr>
<td>Gender (M/F)</td>
<td>18, 6</td>
<td>18, 6</td>
</tr>
<tr>
<td>Yrs employed</td>
<td>5.9 (2.1)</td>
<td>6.5 (2.1)</td>
</tr>
<tr>
<td>FEV1% pred.</td>
<td>86 (51)</td>
<td>97 (7)</td>
</tr>
<tr>
<td>FVC% pred.</td>
<td>103 (11)</td>
<td>102 (11)</td>
</tr>
<tr>
<td>FEV1/FVC%</td>
<td>78 (6)</td>
<td>79 (6)</td>
</tr>
<tr>
<td>B-leucocytes</td>
<td>6.4 (2.7)</td>
<td>6.1 (1.8)</td>
</tr>
<tr>
<td>CRP</td>
<td>1.9 (1.1)</td>
<td>2.3 (4.9)</td>
</tr>
</tbody>
</table>

FEV1 = Forced expiratory flow 1.0 sec, FVC = Forced vital capacity. Data presented as Mean (SD). Data collection 2004-2005, 2007

Inflammation markers in sputum supernatant

Neutrophils

-eosinophils very low values

– 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

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 period
  • Cytokine profile as seen for a general immune response
  • Simultaneously trigging of the specific (antibody mediated) immune defence
    (CD86, CD11b, CD16)

• Inflammation reversible one year after cessation of exposure

Surface markers (flow cyt. analyses)

Conclusions of flow cyt. results:

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

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

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