Novel biomarkers of chemical-induced asthma: a murine model

Jeroen Vanoirbeek
Asthma

1. Chronic airway disease: prevalence: 5-10%, 300,000 people affected world-wide

2. Reversible airflow limitation, airway hyperreactivity & inflammation

- Wheezing
- Shortness of breath
- Coughing
- Chest tightness
Asthma

1. Rapid increase of allergy and asthma in past 40 years

2. Changes in gene pool unlikely

3. Changes in “environment”?  
   - Hygiene, lifestyle, diet, ...
   - Air pollution (indoor, outdoor)
   - Specific chemicals?

4. Mechanisms?
Occupational asthma

1. Definition:
   • occupational asthma is a disease characterized by variable airflow limitation and/or airway hyperresponsiveness due to causes and conditions attributable to a particular occupational environment and not to stimuli encountered outside the workplace

2. 9-15% of all adult asthma is due to exposures on the work floor

3. The most common cause of work-related lung diseases

Bernstein et al., 1999; Mapp et al., 2005
Occupational agents

**High molecular weight (HMW) compounds (≥ 5 kDa)**
- Animal proteins
- Plant proteins
- Enzymes

**Low molecular weight (LMW) compounds (< 5 kDa)**
- Chemicals
- Metals
- Wood dust
- Pharmaca

**Immune sensitization**
- IgE mediated
  - HMW allergens: Flour, Lab animals, ...
- IgE mediated or non-IgE mediated
  - LMW sensitizers: Isocyanates, persulphates, ...

**No immune sensitization**
- Non-IgE mediated
  - LMW irritants: Chlorine, Ammonia, ...
Occupational agents

• Diisocyanates
  ▪ Highly reactive, low molecular weight compounds
  ▪ Most common cause of chemical-induced occupational asthma
  ▪ Used for the production of polyurethanes, foams, paints, etc
Skin - Lung

- Development of chemical-induced asthma
  - Primary route of exposure and initiation of immune response = respiratory tract
  - Regulation and prevention of OA almost exclusively focuses on airborne exposures
  - Despite reduction in workplace respiratory exposure, diisocyanate asthma continues to occur

Focus on skin exposure

Redlich C and Herrick C, Curr Opin Allergy Clin Immunol 2008
Occupational asthma

Mechanisms ?

- Role of chemical properties ?
  - chemical reactivity
  - irritant properties

- Pathways of sensitization ?
  - via dermal route ?

- Immunological mechanisms ?
  - usually no specific IgE antibodies
  - cellular mechanisms

Implications

- Hazard identification
  - Prediction of asthmogens

- Prevention
  - Avoid skin contact

- Surveillance & diagnosis
  - Identification of sensitized subjects
  - Identification of sensitizing agent in affected subjects
Aim

To identify (early) biomarkers of chemical-induced asthma and sensitization to chemicals using proteomics techniques
Proteomics

• Proteomics is a global strategy in which all proteins (the proteome) derived from a cell, tissue, body liquid or whole organism, are simultaneously visualized and identified
Two-dimensional difference gel electrophoresis: 2D-DIGE
Two-dimensional difference gel electrophoresis: 2D-DIGE

pH = 3

pH = 10
Two-dimensional difference gel electrophoresis: 2D-DIGE
Two-dimensional difference gel electrophoresis: 2D-DIGE
Two-dimensional difference gel electrophoresis: 2D-DIGE
Two-dimensional difference gel electrophoresis: 2D-DIGE

Trypsin Digest

MALDI-TOF MS

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Proteomics in chemical-induced asthma

• Classical research: focus on immune related cells and cytokines, resulting in many insights, but exact mechanisms of OA still unclear

• Development of new & sensitive methods → new approaches
Proteomics in chemical-induced asthma

Day 1

Dermal treatment on both ears (20 µl) with 0.3%TDI or AOO (vehicle)

Day 8

sensitization

Vanoorbeek et al., 2003, 2004, 2008; De Vooght et al., 2010
Proteomics in chemical-induced asthma

Day 1

Dermal treatment on both ears (20 µl) with 0.3% TDI or AOO (vehicle)

sensitization

Day 8

Airway challenge: 0.01% TDI or AOO (vehicle)

challenge

Day 15

Vanoirbeek et al., 2003, 2004, 2008; De Vooght et al., 2010
Proteomics in chemical-induced asthma

Day 1

- Dermal treatment on both ears (20 µl) with 0.3% TDI or AOO (vehicle)

Day 8

- Airway challenge: 0.01% TDI or AOO (vehicle)

Day 15

- AHR (methacholine)

Sample collection:
- auricular lymph nodes
- serum
- BAL

Vanoirbeek et al., 2003, 2004, 2008; De Vooght et al., 2010
Proteomics in chemical-induced asthma
Proteomics in chemical-induced asthma

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<td><strong>BAL</strong></td>
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<td><strong>Serum</strong></td>
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All differences are significant at p < 0.01.
Proteomics in chemical-induced asthma

Spot Maps (Score Plot)

Auricular lymph nodes
Proteomics in chemical-induced asthma
Proteomics in chemical-induced asthma

- auricular lymph nodes
- bronchoalveolar lavage
- serum
Proteomics in chemical-induced asthma

- Lymphocyte specific protein-1

- Literature:
  - Expression:
    - lymphocytes
    - neutrophils
    - macrophages
  - Chemotaxis & activation neutro
  - Overexpression → dysfunction
  - Regulated by IL-4

Vanoirbeek et al., 2008, De Vooght et al., 2009
Conclusions

1. First systematic & systemic proteomics approach in a model of occupational asthma

2. Dermal sensitization and a single airway challenge leads to profound proteome changes in multiple compartments

3. Physiological and immunological changes are reflected by changes in the proteome
Proteome changes in auricular lymph nodes and serum after dermal sensitization to toluene diisocyanate in mice
Proteome changes after dermal sensitization

1. So far, no study has focused on early time points

2. Human (early) biomarker research is complicated: only when disease has established
Proteome changes after dermal sensitization

1. So far, no study has focused on early time points

2. Human (early) biomarker research is complicated: only when disease has established

3. Rationale: investigate changes in the proteome during sensitization to identify (early) markers of sensitization
Proteome changes after dermal sensitization

Day 1

- Dermal treatment on both ears (20 µl) with 0.3% TDI or AOO (vehicle)
- Sensitization

8

- Oropharyngeal aspiration: 0.01% TDI or AOO (vehicle)
- Challenge

15 16

- AHR (methacholine)
- Sample collection:
  - Auricular lymph nodes
  - Serum

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Proteome changes after dermal sensitization

Auricular lymph node weight
Proteome changes after dermal sensitization

Lymphocyte subpopulations

1 application

2 applications
2D-DIGE workflow

1. Labeling
2. Separation
3. Scanning
4. Decyder analysis
5. Mass spectrometry
6. Identification
Proteome changes after dermal sensitization

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Proteome changes after dermal sensitization

auricular lymph nodes

1 application

2 applications

[Graph showing proteome changes with 1 and 2 applications, labeled AOO and TDI]
Proteome changes after dermal sensitization

serum

1 application

2 applications

- AOO
- TDI
Proteome changes after dermal sensitization

auricular lymph nodes

1 application
- metabolism: 11%
- stress response: 15%
- protein folding: 12%
- structural: 27%
- others: 27%
- binding/transport: 4%
- immune response: 4%

2 applications
- metabolism: 20%
- stress response: 20%
- protein folding: 9%
- binding/transport: 3%
- immune response: 8%
- structural: 20%
- others: 20%
Proteome changes after dermal sensitization

serum

1 application
- binding/transport: 67%
- metabolism: 33%

2 applications
- binding/transport: 50%
- metabolism: 30%
- protein folding: 10%
- stress response: 10%
Proteome changes after dermal sensitization
Proteome changes after dermal sensitization

- Lymphocyte specific protein-1

- Literature:
  - Expression:
    - lymphocytes
    - neutrophils
    - macrophages
  - Chemotaxis & activation neutro
  - Overexpression → dysfunction
  - Regulated by IL-4
  - Down regulated in complete model (Haenen et al., 2010)
Proteome changes after dermal sensitization

LSP-1

1 application

2 applications

ratio LSP1/GAPDH

AOO TDI

0.0

0.1

0.2

0.3

0.4

0.5

0.6

0.7

0.8

0.9

1.0

LSP-1

GAPDH

***
Proteome changes after dermal sensitization

- Coronin 1a

- Literature:
  - Member of well-conserved family of coronin proteins
  - Mediates Ca$^{2+}$ release
  - Involvement T cell activation and proliferation
Proteome changes after dermal sensitization

Cor 1a

1 application

2 applications

ratio Cor1a/GAPDH

**

***

**

AOO TDI

GAPDH

Cor1a

Proteome changes after dermal sensitization
Proteome changes after dermal sensitization

- **Hemopexin**
- acute phase protein
- **Literature:**
  - Involved in innate body defense
  - Upregulated during inflammation
  - Also in complete model (BAL and serum)
Proteome changes after dermal sensitization

Hemopexin

1 application

2 applications

mg/ml
Conclusions

1. Sensitization causes profound differences in the proteome of TDI-sensitized mice compared to control mice

2. A subset of proteins were confirmed in an independent set of mice

3. Validation needed in human exposed workers
Acknowledgements

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