

## **Novel biomarkers of chemical-induced asthma: a murine model**

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**Introduction.** Occupational asthma is the principal cause of work-related respiratory disease in the industrial world. Toluene-2,4-diisocyanate (TDI) is one of the most common respiratory sensitizers leading to occupational asthma. Using a mouse model (male BALB/c mice, 6 weeks old, 20g) of chemical-induced asthma, we explored proteome changes in multiple compartments of mice sensitized and challenged with TDI or acetone-olive oil (AOO; vehicle).

**Methods.** Airway reactivity to methacholine and a bronchoalveolar lavage (BAL) cell count was assessed in treated and control mice, 1 day after challenge. Subsequently, two-dimensional differential gel electrophoresis (2D-DIGE) was performed on auricular lymph nodes, BAL, and serum comparing TDI-treated and vehicle-treated control mice. The differentially expressed proteins were identified by mass spectrometry and pathway analysis was performed.

AHR data were analyzed using a two-way ANOVA, while the AUC of *R* was analyzed with a Student's *t*-test. The differential cell counts were analyzed with a non-parametric Mann-Whitney test (Graphpad Prism 4.01, Graphpad Software Inc, San Diego, USA). A level of  $p < 0.05$  (two-tailed) was considered significant. Differentially expressed proteins were matched and analysed (Student-*t*-test) by the Decyder 7.0 software package.

**Results.** TDI-treated mice exhibit increased airway reactivity (2.6-fold increase) and a neutrophilic inflammation in the BAL fluid, compared to control mice. 2D-DIGE showed 53, 210, and 40 differentially expressed proteins in the auricular lymph nodes, BAL, and serum of TDI-treated versus vehicle-treated mice, respectively. Several of the identified proteins could be linked with inflammation, neutrophil chemotaxis, and/or oxidative stress.

**Conclusion.** Physiologic and immunologic readouts of the asthmatic phenotype, such as inflammation, were confirmed in three compartments by several of the differentially expressed proteins via 2D-DIGE and computerized pathway analysis.

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