

Network: Diseases Due to Environmental Factors**Project: Functional Evaluation of Newly Identified Genes and Polymorphisms Relevant for Bronchial Asthma in a Murine Model of Allergen-induced Airway Disease**

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Introduction

The systemic analysis of genetic variants in their functional context will allow to finally understand the contribution of genetic predisposition to the development of bronchial asthma and other atopic disorders. To reach this goal, a close interaction between population-based studies and functional analysis in animal models has to be accomplished. In this context, the utilization of an animal model allows the systematic analysis of gene-phenotype interactions and differential gene expression in the course of disease development and progression in a genetically homogeneous population and with full control of environmental influences. The aim of this project is to increase the understanding of disease mechanisms by identifying new genes and functional relevant polymorphisms for disease development utilizing a murine model of bronchial asthma. The integration of the murine model in the concept of genetic analysis of bronchial asthma will allow us to extend observations from studies in human tissues to organs or cells that are not available in patients, to identify common mechanisms of disease development, to establish chronic disease models that closer resemble the features of allergic disease in patients, to identify new genes and relevant polymorphisms involved in the development of bronchial asthma, to functionally evaluate the role of newly identified biomarkers/ genes / polymorphisms from human and murine studies for disease development and progression, and to finally establish novel strategies for better prediction, prevention and treatment of bronchial asthma.

Project Status**Murine model of bronchial asthma**

We have established a murine model of allergen-mediated airway disease that offers the unique opportunity to study *in vivo* allergen sensitization in a genetically homogenous population with a clearly defined disease phenotype and with full control of environmental influences. This model comprises the main feature of the disease: (I) increased production of allergen-specific IgE following systemic or inhalative sensitization with allergen; (II) marked inflammatory responses in lung tissues, and (III) development of unspecific airway hyperreactivity (AHR) detectable by *in vivo* lung function measurements, following allergen airway challenges of sensitized mice. We have extensively studied immunological mechanisms and environmental conditions leading to disease onset and progression and identified crucial factors for the development of murine bronchial asthma (1-10).

Gene expression profiling

Utilizing the murine asthma model, we analyzed gene expression profiles with oligonucleotide array hybridization techniques in different cell compartments and at different time points of the allergen sensitization and challenge protocol to delineate the genetic regulation of allergen-induced sensitization and airway inflammation on the different levels of the disease: allergen-induced immune reactions (T-cells, B-cells from spleen and peribronchial lymph nodes), inflammatory responses in the target organ (lung tissues) and changes of airway function (parasympathic ganglia).

We were able to:

- Identify more than 1000 differentially expressed genes during the progression of allergen-mediated airway disease.

- Identify gene expression profiles specific for certain compartments of disease manifestation: spleen, local lymph nodes, lung tissues, neuronal ganglia.

- Identify gene expression profiles specific for certain pathophysiological aspects: allergic sensitization, airway inflammation, development of AHR.

- Identify novel genes with functional relevance for the development of allergen-induced airway inflammation (11,12).

Functional evaluation of novel biomarkers

One of the most significant benefits of using a murine model is that it allows a direct functional evaluation of novel genes and biomarkers. A good example for successful identification of a new biomarker with functional importance for disease development utilizing our approach is the signal-transducer and activator-of-transcription factor (STAT-1) (12). STAT-1 expression by epithelial cells in lung tissues of sensitized and challenged mice is upregulated and may be critical for the development of inflammatory responses and AHR following airway allergen challenges of previously sensitized mice. For functional analysis, we utilized a novel approach to inhibit STAT-1-mediated gene expression by applying a decoy technique. This approach resulted in:

- Attenuated leukocytic airway inflammation

- Decreased Th2-cytokine production in lung tissues

- Reduced expression of adhesion molecule VCAM-1 by endothelial cells and of costimulated antigen CD40 by epithelial cells

- Inhibition of the development of *in vivo* AHR

STAT-1 (and other newly identified factors with functional properties for disease development, like inducible co-stimulator, ICOS) may thus serve as a novel diagnostic or therapeutic target for disease prediction and/or prevention.

Results

Early prediction and effective prevention of allergic diseases requires a better understanding of the genetic program underlying the pathophysiological mechanisms. In order to evaluate the molecular changes initiated by allergen sensitization and airway challenges in a murine model of bronchial asthma, gene expression profiles were analyzed by microarray techniques (Fig 1+2) in two different mouse strains (BALB/c, C57/Bl6) and four different organs (lung, spleen, lymph nodes and ganglia), which are involved in the development of the allergic phenotype. Comparative analysis of expression profiles of lung tissues in the early phase of airway inflammation following allergen sensitization and airway challenges revealed that a total of 1,1% of all genes were upregulated compared to sensitized and sham-challenged animals. Two of these genes had so far been described in oxidative stress responses, yet, their particular role in the development of allergen-induced airway inflammation remains indecisive. Therefore, we further delineated the involvement of the novel genes and confirmed the microarray results via quantitative PCR (Fig 3+4). Western blot analysis and immunohistology showed that RNA-upregulation translated into enhanced protein production and distinct expression patterns in the bronchial epithelium for both genes. The data suggest that these genes represent novel and common inflammatory genes involved in early stages during the development of allergen mediated airway

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disease.

Consequently, these genes are currently tested for their functional relevance for the occurrence of allergic asthma and may ultimately provide new targets for the diagnosis or treatment of the disease.

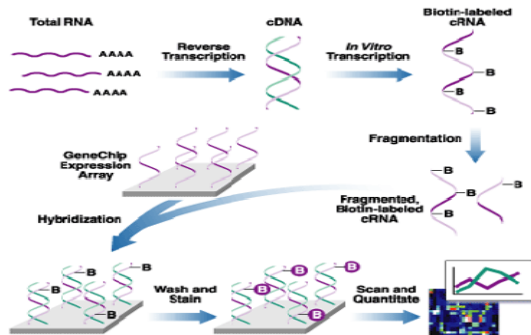


Fig 1: RNA expression profiles of lung tissue was examined by using a murine **Affymetrix** chip (U74 A), which represents ~12000 already annotated mouse genes.

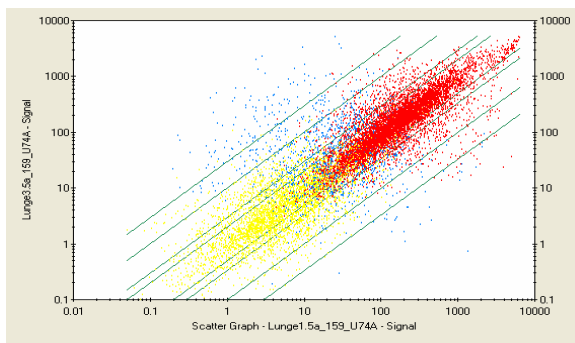
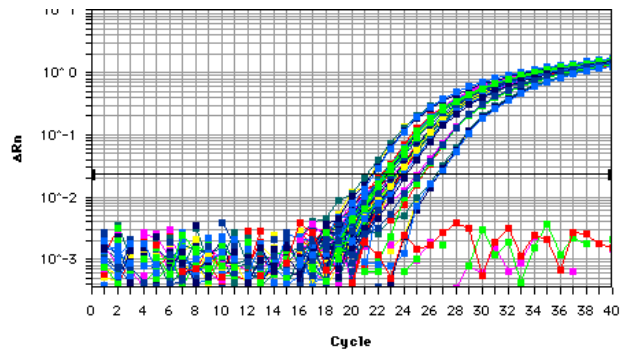


Fig 2: Scatter plot of expressed genes, comparing naïve mice and animals with allergen induced airway inflammation.



Fig 3: Qualitative verification by PCR

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- Lit.: 1. Hamelmann E, Takeda K, Haczku A, Cieslewicz G, Shultz LD, Hamid QA, Xing Z, Gauldie J, and Gelfand EW. IL-5 but not IgE reconstitutes airway inflammation and hyperresponsiveness in IL-4 deficient mice. *Am.J Respir. Cell Mol.Biol.* 2000;23:327-334. 2. Haczku A, Takeda K, Hamelmann E, Loader J, Joetham A, Irvin CG, Lee JJ, Kikutani H, Conrad D, Gelfand EW. CD23 Exhibits Negative Regulatory Effects on Allergic Sensitization and Airway Hyperresponsiveness. *Am. J. Respir. Crit. Care Med.* 2000;161 952-960. 3. Hamelmann E. Anti IgE und Anti-IL-5_ Neue therapeutische Konzepte für die Behandlung von Allergien. *Allergologie* 2000;12:585-588. 4. Hamelmann E and Gelfand EW. IL-5 induced airway eosinophilia - the key to asthma ? *Immunol. Reviews* 2001;179:182-191. 5. Blümchen K., T. Kallinich, E Hamelmann. Anti-IL-5 Therapy. *Expert Opinion on Biological Therapy* 2001;1(3):433-453. 6. Gerhold K, Blumchen K, Bock A, Seib C, Stock P, Kallinich T, Lohning M, Wahn U, Hamelmann E. Endotoxins prevent murine IgE production, TH2 immune responses, and development of airway eosinophilia but not airway hyperreactivity. *J Allergy Clin Immunol* 2002 Jul;110(1):110-6. 7. Gerhold K, Blümchen K, Bock A, Franke A, Avagjan A and Hamelmann E. Endotoxins and Allergy - Lessons from the Murine Model. *Pathobiology* 2002-2003;70(5):255-9. 8. Löhlein M, Hutloff A, Kallinich T., Radbruch, Hamelmann E, Kroczeck R. Expression of ICOS in vivo defines CD4⁺ effector T cells with high inflammatory potential and a strong bias for secretion of IL-10: *J. Exp. Med.* 2003; 197 (2): 181-193. 9. Kallinich T and Hamelmann E. The role of T cell co-stimulatory signals for the allergic immune reaction. *Allergy* 2004, in press. 10. Quarcoo D and Hamelmann E. Transcription Factors - New Targets for Anti-Allergic Therapy. *Pathobiology* 2003; 70(5):293-6. 11. Quarcoo D, E. Weixler S, Groneberg D, Joachim R, Ahrens B, Wagner AH, Hecker M, and Hamelmann E. STAT-1 is required for the development of allergen-induced airway inflammation and airway hyperreactivity. *J All Clin Immunology* 2004 Aug; 114(2):288-95. 12. Stock P, Kallinich T, Akbari O, Quarcoo D, Gerhold K, Wahn U, Umetsu DT, Hamelmann E. CD8⁺ T cells regulate immune responses in a murine model of allergen-induced sensitization and airway inflammation. *Eur J Immunol* 2004 Jul;34(7):1817-27.