Network: Diseases Due to Environmental Factors

Project: Childhood Asthma: Gene Discovery and Functional Analysis

Michael Kabesch - Ludwigs-Maximilians University, Pediatric Hospital, München - michael.kabesch@med.uni-muenchen.de

Introduction

Asthma is the most common chronic disease during childhood. In Germany, more than 10% of children are affected. Asthma is thought of as an allergic disease, where a non- infectious inflammation of the airways occurs due to a deviation of the immune system. Ubiquitous substances in the environment, such as tree or grass pollen, initialize an over- reaction of the immune system characterized by the influx of eosinophils and the activation of T-helper cells, the production of specific Immunoglobulin E (IgE) antibodies against allergens and the release of histamine from mast cells. These inflammatory mechanisms lead to a swelling of the airway mucosa, the extensive production of mucus and the contraction of bronchial muscles. In turn, airway obstruction occurs and patients have difficulties to breath. Furthermore, these inflammatory responses may alter the barrier function of the airway epithelium and additional immunological reactions against new allergens may follow.

It has been suggested that genetic predisposition and environmental factors both influence the development of the disease. The genetic contribution in asthma and allergy was estimated to be 70% in twin studies. In asthma, as in many other complex diseases, it is not a single gene alteration causing the disease but a number of different genetic alterations add to the development of the disease. Genetics may determine the possible direction and the strength of the immune reaction. Genes involved in the barrier function of the lung epithelium could also play a role in the primary induction of the disease. Alterations in these genes may contribute to the deviated recognition and an impaired signalling following the encounter with environmental agents on the surface of the airways. After these first steps of gene environment interaction, a number of immunological feedback loops will start or alternatively, be suppressed. Here, the innate as well as the adaptive immune system are involved and may interact at different levels. The aim of this NGFN project was to study these genetically influenced interactions between the airways and the environment and to identify gene by gene interactions contributing to the development of asthma during childhood.

Results

To discover genes involved in the pathogenesis of asthma, large scale association studies were performed in a cross sectional German population sample. For this purpose, more than 5,000 DNA samples from the German leg of the International Study of Asthma and Allergy in Childhood (ISAAC phase II) were collected in Munich, Leipzig and Dresden. All children were thoroughly phenotyped for asthma and other atopic diseases. In this population- one of the largest asthma and atopy DNA collections world- wide- genes involved in different aspects of asthma development were analysed in multiple projects.

Innate immunity genes and the environment

Innate immunity genes interact with the microbial environment and direct the early phase response to microbial stimuli (**1**). This system comprises extra cellular and intracellular pathogen recognition receptors as well as downstream signalling molecules as shown in a simplified sketch (figure 1). The innate immune system interacts on different levels with the adaptive immune system activating specific T helper cells and inducing immunoglobulin dependent effector systems, such as B-cells and mast cells. It has been hypothesised that alterations in this system may deviate the immune response from a bacterial and viral focused reply towards an allergen inducible response.

Thus, we have studied genetic alterations in some central pattern recognition molecules such as CD14, CARD15 (previously NOD2) and CARD4 (previously NOD1) for their role in the development of asthma and atopy. In these projects it could be shown that polymorphisms in these genes (a) influence the expression of specific receptors without an impact on atopic diseases (CD14) (**2**) or (b) lead to an increased risk for atopic diseases such as allergic rhinitis, atopic eczema and asthma (NOD 1 &2) (**3,4**). All these results have so far been replicated in independent populations. Of interest, some of the atopy associated polymorphisms in the NOD2 gene have previously been identified as risk genes for Crohn's disease by another partner within the NGFN network for environmental diseases (see also project inflammatory bowel disease, Kiel).

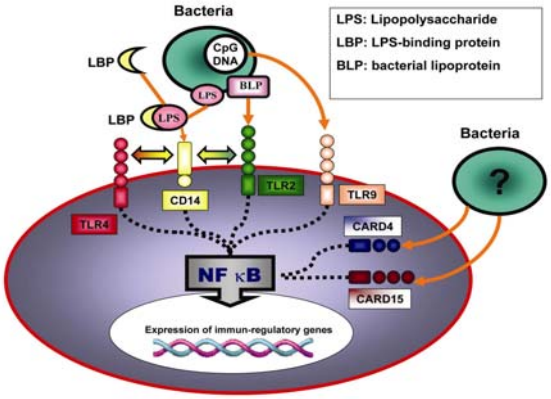

Fig 1: *Innate pathogen recognition receptors interact with microbial compounds influencing the immune response*

Passive smoking, detoxification genes & asthma

Another focus of the asthma research project was to study gene environment interactions. That genetic and environmental factors both influence the disease seemed clear but very little was so far known on how these interactions may occur. To study this phenomenon, we investigated the effect of passive smoke exposure, one of the major and undisputed environmental risk factors contributing to the development of asthma. We hypothesised, that genetic alterations in detoxification enzymes may modulate the susceptibility to develop asthma in children exposed to high amounts of cigarette smoke. A major group of detoxification enzymes in humans are the glutathione S transferase (GST). Some of these enzyme classes are missing in a large percentage of Europeans due to gene deletion effects. Thus, approximately 50% of German children are missing the GST M1 gene and 15% are missing the GST T1 gene (and the subsequent detoxification enzymes). Investigating more than 3.000 children we could show that a concomitant deficiency of GST enzymes and the exposure to passive smoke dramatically increases the risk for asthma, asthma symptoms and disease severity. Furthermore, lung function was impaired in children with GST deficiency whose mothers smoked during pregnancy. All these adverse health effects were most pronounced when both the genetic risk genotype and the environmental exposure where present concomitantly. The statistical analysis suggested strong interaction effects (**5**).

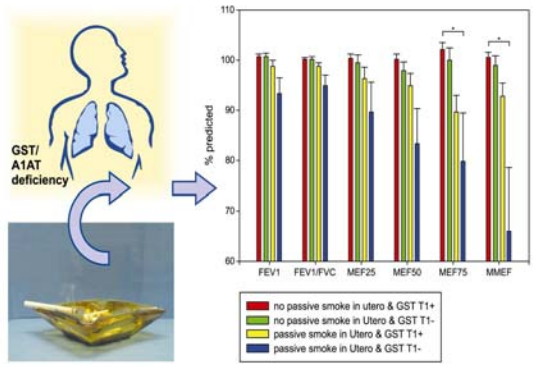

Fig 2: *Prenatal passive smoke exposure and genetic deficiency of detoxification enzymes impair lung function measurements in children (blue bars).*

Pathway genetics in asthma

Not only genes and environments interact in the development of atopic diseases but also genes with genes. Different genes contribute to the development of "atopy syndromes"; some acting quite independent from each other, while others may be connected in signalling pathways. One of the most intriguing signalling pathway in atopy is the interleukin 4/ interleukin 13 pathway. From immunology it has long been known that interleukin 4 is the central cytokine involved in immunoglobulin E (IgE) switching in B-cells, a cornerstone common to all atopic diseases. Only recently it has been detected, that a second cytokine closely related to IL-4, named IL-13, is also a potent mediator of atopic immune responses very likely even more potent in the induction of asthma than IL-4. Both IL-4 and IL-13 are produced by the central atopy cell, the T regulatory cell. Both cytokines signal through a common pathway including a shared receptor chain (IL4Ra) and an intracellular signal transducer (STAT6) as shown in figure 3.

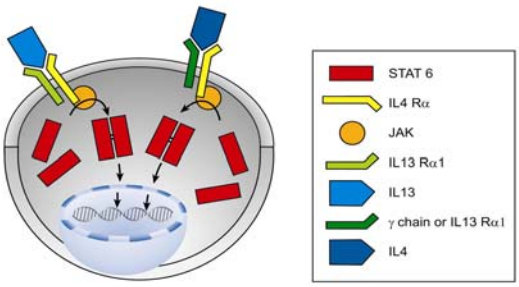

Fig 3: *Interleukin 4 and 13 share a common signaling pathway. Genetic alterations of this pathway contribute significantly to the regulation of IgE levels and atopy.*

As this pathway is so important for the regulation of IgE we hypothesised that alterations in any central component of the pathway may increase the risk to develop atopic diseases. We systematically investigated the effect of polymorphisms in the 4 major genes of the pathway: IL-4, IL-13, IL4Ra and STAT6. Where no data on polymorphisms were available, we performed whole gene mutation screens in representative sub-samples (IL-4, IL-13). As hypothesised, polymorphisms in all 4 pathway genes led to increased IgE levels and the development of atopic diseases in our population (**6,7,8,9**). Furthermore, we investigated gene by gene interaction in the IL-4/IL-13 pathway presuming that the concomitant presence of more than one genetic alteration in the pathway may have more than additive effects. In this analysis, we could show that the risk to develop asthma increased concordantly with the presence of polymorphic genes: From an odds ratio of app. 1.5 if one gene alteration was present to app. 4 fold in the presence of 2 polymorphic genes and to an odds ratio of app. 16 fold in the presence of specific combinations of 3 polymorphic genes in the pathway. From a statistical, population-genetics approach these data indicate a gene by gene interaction in the IL-4/IL-13 pathway resulting in a significantly increased risk for asthma as well as elevated serum IgE levels. However, true gene by gene interaction also needs to be demonstrated on the functional level. Thus, functional studies of the putatively relevant polymorphisms in the IL-4/IL-13 pathway are now performed. Interactions of these polymorphic or alternatively regulated genes are now investigated on a molecular level.
Based on these results, it can be speculated, that a proportion of the population may be identified by genetic testing to be at high risk to develop atopic diseases. If gene by environment interaction are involved, as it seems to be the case with GST deficiency and passive smoke exposure, targeted preventive measures may be applicable based to these tests in the population.

Outlook

So far, many pieces of the asthma-genetic jigsaw have been identified and this project has contributed to this large worldwide quest. National and larger than national collaborations are necessary to answer some of the remaining questions. This lead to the formation of a special national research focus (SFB) on pulmonary allergy supported by the German research council (DFG) and a large scale integrated project on gene by environment interaction in asthma by the European Community. This NGFN2 project is embedded in both scientific programs.
In these projects as well as in the NGFN2 project, the future focus is on linking different asthma susceptibility genes and pathways with each other. The effect of epigenetic and environmental influences will be studied and the functional role of genetic alterations and polymorphisms will be investigated. Using animal and human in vitro models, the function of polymorphisms will be elucidated. Furthermore, large scale studies will allow for genome wide association studies identifying new asthma genes.
Taken together, new disease models will be established leading to a new and better understanding of atopic diseases affecting millions of children in Germany and many more around the world. Finally, this will be the basis of new diagnostic and therapeutic approaches and the cornerstone of more effective disease prevention strategies in the near future.

Lit.: ***1.*** *Kabesch M et al. Why Old McDonald had a farm but no allergies: genes, environments, and the hygiene hypothesis. J Leukoc Biol. 2004 Mar;75(3):383-7.* ***2.*** *Kabesch M et al. A promoter polymorphism in the CD14 gene is associated with elevated levels of soluble CD14 but not with IgE or atopic diseases. Allergy. 2004 May;59(5):520-5.*
3. *Kabesch M et al. Association between polymorphisms in CARD15 and allergy in two German populations. J Allergy Clin Immunol. 2003 Apr;111(4):813-7.* ***4.*** *Hysi P et al. NOD1 variation, immunoglobulin E and asthma.Hum Mol Genet. 2005 Apr 1;14(7):935-41.* ***5.*** *Kabesch M et al. Glutathione S transferase deficiency and passive smoking increase childhood asthma.Thorax. 2004 Jul;59(7):569-73.* ***6.*** *Graves P et al. A cluster of seven tightly linked polymorphisms in the IL-13 gene is associated with total serum IgE levels in three populations of white children.J Allergy Clin Immunol. 2000 Mar;105(3):506-13.* ***7.*** *Kabesch M et al. A complete screening of the IL4 gene: novel polymorphisms and their association with asthma and IgE in childhood.J Allergy Clin Immunol. 2003 Nov;112(5):893-8.* ***8.*** *Schedel M et al. A signal transducer and activator of transcription 6 haplotype influences the regulation of serum IgE levels.J Allergy Clin Immunol. 2004 Nov;114(5):1100-5.* ***9.*** *Huehn J et al. A signal transducer and activator of transcription 6 haplotype influences the regulation of serum IgE levels.J Allergy Clin Immunol. 2004 Nov;114(5):1100-5.*