

**PROTECTION AGAINST ALLERGY: STUDY IN RURAL ENVIRONMENTS (PASTURE)**

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The overall aim of the project was to assess the role of elevated levels of indoor exposure to microbial products for the development of childhood allergies in a prospective study in rural environments across Europe. Furthermore, this project investigated the immunological and genetic mechanisms involved in the determination of individual responses to these environmental influences. The results of this study will guide the further development of preventive strategies in Europe using microbial products in subjects with genetically determined responsiveness to microbial matter.

### **Results and Milestones:**

We performed a large multicentre birth cohort study in five European countries. In total, 1,163 children were recruited into the study. At age 1 year, 1,068 were still included which amounts to an excellent follow-up rate of 91.8%. Extensive questionnaire data about both parents, in particular about maternal exposures and health outcomes during her pregnancy, as well as about the study child's sibling(s) was collected. Furthermore, many different exposures to farming environments, but also to other lifestyle factors such as for example nutrition have been assessed via questionnaires. In addition, environmental dust samples and cow's milk samples were collected and analysed. Last not least, extensive immunological and genetic data were obtained from the study child at birth as well as at age 1 year, as well as from both parents. The response rate at birth for stimulation assays and cytokine measurements amounted to almost 70%, for measurements of cord blood IgE to 80%, and for genotyping to 87%.

We confirmed the protective farm effect seen in previous cross-sectional studies in the PASTURE birth cohort as the parents' and siblings' prevalence rates of allergic illnesses were significantly lower among farming families than in non-farming controls. These findings clearly demonstrate that we selected an informative population for our study. The 'farm effect' may first be detected in immune responses at birth. Therefore, we investigated the cytokine responses in cord blood after stimulation with LPS, SEB and PMA/Ionomycin in farm and non-farm children. We did find differences in INF-gamma and TNF-alpha secretion between farm and non-farm children at birth. Both cytokine levels were increased after stimulation with PMA/Ionomycin among the farm children. When investigating the factors underlying this "farm effect", we could demonstrate that maternal exposure to farm animals and exposure to barns (hay lofts) was responsible for the difference in cytokine levels in cord blood between farm and non-farm children. Interestingly, offspring of mothers who were not living on a farm, but had contact to farm

animals during pregnancy, also showed increased INF-gamma and TNF-alpha levels after stimulation with PMA/Ionomycin. Levels of IL-10 secretion after stimulation were not affected by the farming status suggesting that the protective effect may be mediated by factors other than those associated with T-regulatory cells.

Immune responses at birth also comprise the production of IgE antibodies to a number of food and inhalant allergens. Serum-IgE was measured by a sensitive assay in cord blood samples of children participating in the PASTURE birth cohort. Cord blood IgE antibodies to outdoor allergens, in particular to grass pollen, were less prevalent among farmers than among non-farmers, while farm children had a higher prevalence of IgE antibodies to food allergens than non-farm children in their cord blood. There was an association between IgE-levels in cord blood and levels of specific IgE antibodies in the parents' sera. Correlations with maternal levels were stronger than those with paternal levels suggesting that the levels in cord blood are not exclusively determined by genetic factors. Contamination of cord blood samples by maternal blood had been excluded by measurements of IgA in the offspring's samples.

From most children and parents, DNA was extracted from EDTA-blood samples, and genotyping of a vast number of SNP's in 10 Toll-like receptors has been performed successfully. All cord blood RNA samples have been extracted and transformed into cDNA. Analyses of these samples are ongoing.

Environmental samples from house dust and cow's milk were also taken. First analyses of house dust samples show that levels of microbial components and of allergens are linked to the farming status. As expected, endotoxin as well as EPS concentrations were higher in farm households than in non-farm households. These exposure data will be related to immune responses at birth and at age 1 year as well as to disease manifestations until age 1 year. Measurements of endotoxin have also been performed in the cow's milk samples. Unexpectedly, endotoxin concentrations were higher in non-farm samples than in farm samples, and higher in milk bought in the shop than in non-pasteurised farm milk.

### **Benefits and Beneficiaries:**

This study is in a unique position to investigate a number of protective factors for the development of childhood asthma and allergies in Europe. The acquired large data set will allow an investigation of interactions between environmental exposures and a child's genetic susceptibility to these exposures. Furthermore, through the extensive immune and gene expression studies we can investigate various mechanisms underlying these protective 'farm effects' early in life. We will particularly focus on maternal - offspring interactions. By investigating these various interactions between maternal and filial factors, between environment and genes, and between gene expression and immune responses we will eventually contribute to a better understanding of these complex interrelationships. An improved understanding will eventually allow the development of novel prevention strategies focussing on changes in environmental conditions and/or on development of novel drugs mimicking natural exposures, which will be applicable to all European countries. By considering the genetic determinants of responses to such exposures and drugs, respectively, individually targeted recommendations will eventually be issued. By the implementation of successful preventive strategies, health care costs can be reduced dramatically. In principle, a reduction in the prevalence of allergy and asthma to 20% - 35% of baseline figures can be achieved if natural exposures occurring in early life in farming environments can successfully be imitated.

The findings of this study will contribute to improve the quality of life among children and their families by developing strategies for the primary prevention of childhood allergies. The results will furthermore contribute to improvements of indoor environments necessary for the health and well-being of families.

