



Mechanisms of early protective exposures on allergy development

The collaborative FP7 EU-project - EFRAIM (KBBE-2007-2-2-06)



Dear Reader,  We would like to introduce you to our EFRAIM project. This project is funded by the European Commission. It prospectively investigates protective factors in early life influencing the development of allergies in birth cohorts conducted in rural areas in five European countries. Detailed information on a great variety of environmental exposures is being collected and large biobanks have been established. The mechanisms mediating these protective exposures such as the maturation of immune responses, gut colonisation, the mucosal barrier function and the genetic and epigenetic factors interacting with the environmental exposures are investigated.	Table of contents:																			
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**Background:**

Allergy has developed into a major health concern in Europe with over 80 million people affected by some form of allergic disease and with around 30 million people suffering from asthma. Allergic diseases can currently be managed effectively but not cured. They diminish patients' quality of life and have considerable socio-economic costs, such as health care utilization, medication, and school or work days missed. More and more people are developing allergic diseases and it is estimated that, by 2015, one in two Europeans is likely to suffer from at least one form of allergy ([www.ga2len.net](http://www.ga2len.net)).

The incidence of allergic diseases is highest early in life. IgE antibodies directed against dietary allergens can already be detected shortly after birth. There is a progressive increase in the incidence of atopic sensitisation over childhood and adolescent years, yet the best predictor for later incidence is manifest atopic sensitisation in the first 3 years of life [1]. Atopic eczema is manifesting in the first year of life in a significant proportion. Likewise, most children with asthma start wheezing in the first 1-3 years of life. However, not all children wheezing in the first years of life will go on to develop asthma. Only a prospective assessment of symptom progression with objective measures of lung function and airway inflammation will allow the identification of childhood asthma cases with reasonable certainty.

Any dietary, life style or environmental determinant of allergic disease must therefore occur before the first manifestation of illness and before the conclusive maturation of the immune

system. Environmental exposures such as the living environment, dietary habits, or microbial exposures are likely to play a significant role when occurring early in life or during pregnancy. Allergic diseases are believed to be determined by multiple factors, including gender, race and genetic predisposition. A number of protective dietary and environmental candidate exposures have been identified in cross sectional surveys, but their causal contribution cannot be assessed with certainty, because the temporal relation between exposure and onset of illness remains ill defined. Several studies across rural areas in Europe have consistently demonstrated that children raised on farms are less likely to develop atopic sensitization, hay fever, wheeze and asthma. Although different sources of protection within the farm environment, such as consumption of raw farm milk, exposure to livestock, and some animal fodder may confer protection, the exact nature of the biologic substances involved in protection are still unknown.

Furthermore, the mechanisms by which such exposures impact on a child's immune response ultimately resulting in aberrant and exuberant reactions towards normally well tolerated environmental exposures remain largely unknown. Therefore, only birth cohort studies across rural areas in Europe enrolling children during pregnancy and including investigations of mechanistic pathways will allow adequate evaluation of exogenous factors for the incidence of allergic diseases by linking the exposure to the development of allergic illness in a given child.

A number of cross-sectional studies carried out in rural areas in Europe have shown that children raised on farms develop less allergic diseases. However, the timing of exposures matters. Therefore, a prospective birth cohort was started in rural areas of Europe.

### **The study design:**

The birth cohort was initially called PASTURE cohort. Recruitment and the follow up until age 1 was funded by the FP6-EU-PASTURE contract (QLRT-2001-00250). Between 2002 and 2005 five study centres in Austria, Germany, Finland, France and Switzerland enrolled over 1,000 children born to farm and non-farm women. The presence of asthma and allergic illnesses was assessed in the parents before their child's birth and in the children at birth, at age 2 and 12 months. Particular attention was given to environmental, microbial and dietary exposure as well as to the child's maturing immune response early in life. In addition genetic factors were taken into account.

In part supported by the FP6- FORALLVENT EU-project contract (SSA. Contract no. 3170), and in part by the 5 field centres own funding, the contact to the cohort was maintained by follow up questionnaire assessments at age 1.5, two, three, four and five years. Furthermore, environmental samples for microbial and dietary determinants were collected. Maturation of the immune system was again investigated in blood samples collected at age 4.5 years.

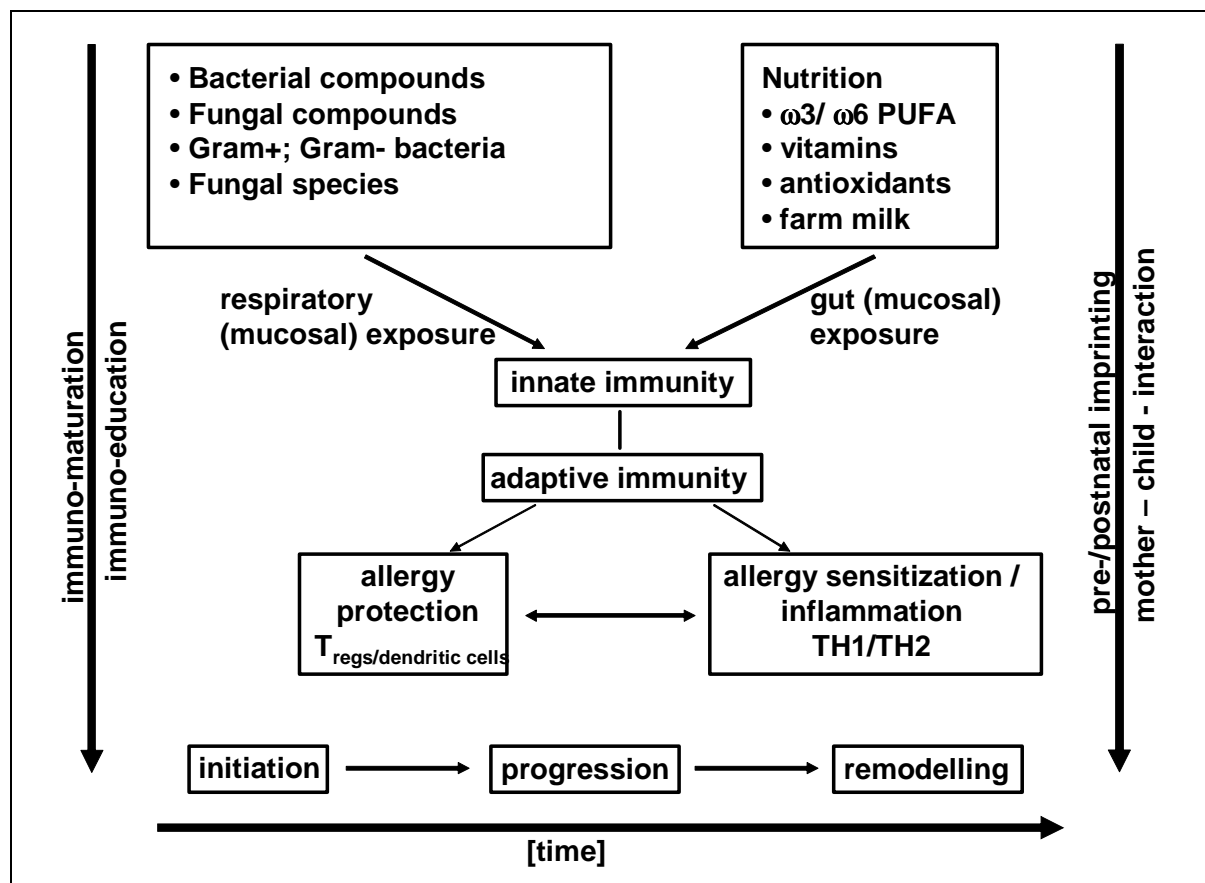
EFRAIM is the continuation of follow up of the PASTURE birth cohort until age 6. The assessment includes a comprehensive questionnaire on health and environmental exposures. A clinical examination comprises the assessment of the prevalence of atopic dermatitis. Comparable to a routine clinical assessment a lung function test determines respiratory parameters. Concentrations of exhaled nitric oxide are measured to identify inflammatory airway responses in the cohort children. Atopic sensitization is determined as the presence of specific serum IgE antibodies against common inhalant and food allergens. Investigations on the development of the innate and adaptive immune response continue in blood samples of the 6 year olds. These measures include the assessment of gene expression of receptors for the recognition of microbial molecules, the investigation of the differentiation and function of immunologically active blood cells and the assessment of molecules stimulating and

modulating the adaptive immune response. Moreover, environmental samples are collected again for the follow up of microbial and dietary exposures in the cohort.

Assessment of early life factors and the maturation of the immune response:

Many PASTURE samples which had been collected before age 6 have not yet been analysed. The EFRAIM project provides financial resources for the continuation of this work. Early life factors are particularly addressed such as putatively protective factors in breast milk and serum markers of the antioxidant vitamins E and D. Serum levels of  $\omega$ 3- and  $\omega$ 6-polyunsaturated fatty acids and their ratio will be determined and will be related to allergic outcomes. Mucosal barrier function will be measured by different direct and indirect markers. Relevant qualitative and quantitative microbial exposure will be assessed by fingerprinting of faecal, cow's milk and house dust samples collected in the first years of life. Epigenetic phenomena will be analysed in blood samples taken at different time points.

The availability of large numbers of consecutive samples covering immune responses on different levels (receptors, RNA, signalling and modulating proteins, regulating cytokines, immunologically active cells) and genetic information in the five rural birth cohorts allows dissecting the maturation of the immune system under well defined environmental conditions in utero up to school age as shown schematically in the figure below.



### **The Consortium:**

To meet the challenge of this large study, a consortium of experts of several European countries accumulates and shares expertise in the fields of immunology, genetics, microbiology, epidemiology, paediatrics, dust analysis, statistics, milk sciences and vaccine development. It provides laboratory facilities of high standards and up to date methodology.

Prof. Dr. med. vet. Johann Bauer	Technische Universität München	Germany
Prof. Dr. Charlotte Braun-Fahrländer, MD	Universität Basel	Switzerland
Gisela Büchele, MPH	Universität Ulm	Germany
Prof. Jean-Charles Dalphin, MD	Université Franche-Comté	France
Dr. Gert Doekes, PhD	University of Utrecht	Netherlands
Prof. Dr. Urs Frey, MD	Universität Bern	Switzerland
Dr. Ivo Gut, PhD	Commissariat a l'Energie Atomique	France
Prof. Dr. Michael Kabesch, MD	Hannover Medical School	Germany
Dr. Marion Kauth, PhD	Protectimmun GmbH	Germany
Prof. Dr. Roger Lauener, MD	Universität Zürich	Switzerland
Prof. Dr. Erika v. Mutius, MD	Ludwig-Maximilians-Universität München	Germany
Professor Juha Pekkanen, MD	National Public Health Institute	Finland
Prof. Dr. Harald Renz, MD	Philipps-Universität Marburg	Germany
Prof. Dr. Josef Riedler, MD	Kardinal Schwarzenberg'sches Krankenhaus	Austria
Dr. Catherine Stanton, PhD	Teagasc Irish Agriculture and Food Development	Ireland

### **Project Status:**

The EFRAIM project has completed its second year of operation. Fieldwork has progressed as scheduled. Due to effective retention strategies only 12% of the initial cohort had dropped out until school age. Objective assessment of the maturation and adaptation of the immune system has been continued as field work progressed. The respective laboratories have been provided with PASTURE samples and have started analyses in blood, dust and milk samples. RNA has been extracted from all blood samples collected until age 4.5 to study a great number of RNA molecules involved in the maturation of the immune system. Automation of the genome wide approach for epigenetic studies has been elaborated. The methylation status of several asthma candidate genes has been assessed. Investigations on protective mechanisms in the intestinal system are moving forward. Measurements of protective factors in breast milk have been completed.

### **Annual Meeting 2010:**

The 2nd EFRAIM Annual Meeting 2010 took place in the city of Marburg.

The venue was hosted by the Department of Clinical Chemistry and Molecular Diagnostics at Marburg University Hospital, Germany.



On 26 January 2010 the EFRAIM Consortium discussed the progress in the statistical analyses of project data. On 27 January 2010, the Scientific Mid-Term Review was in the focus. The consortium reported the work progress of the past 12 months an external Advisory Board. The Advisory Board critically and constructively evaluated the project progress.

### **The Advisory Board:**

The Advisory Board consists of ten members: scientific experts, as well as representatives from biotechnological and pharmacological industries, from public health authorities, and from patient interest groups. It evaluates the project progress and reports to the EC:

Dr. Enrique Fernandez-Caldas	Applied Pharmacological Immunology	Immutek SL, Madrid, Spain
Dr. Koenrad Duhem	Epidemiology and Dust Analysis	CNIEL, Paris, France
Prof. Tari Haatela	Clinical Allergology	Skin and Allergy Hospital, University Helsinki, Finland
Prof. Dick Heederik	Risk Assessment Sciences, i.e. House Dust Compounds	Utrecht University, Netherlands
Prof. Francine Kaufmann	Epidemiology and Biostatistics	INSERM, Paris, France
Otto Spranger	Member of the Austrian Lung Union and the European Federation of Allergy and Airways Diseases	Vienna, Austria
Prof. Dominique Vuitton	Immunology	Université Franche-Comté, Besançon, France
Dr. Karl-Heinz Wiesmüller	Applied Molecular Immunology	EMC Microcollections GmbH, Tübingen, Germany
Prof. Manfred Wildner	Public Health	Bavarian Governmental Agency for Health and Food Safety, Munich, Germany
Prof. Maria Yazdanbaksh	Parasitology, Immunology	Leiden University, Netherlands

## **Results:**

A number of studies have been carried out in rural areas in Europe [2] to contrast the prevalence of asthma and allergic diseases in children living on farms as compared to subjects also living in rural areas but not on farms. Almost all studies reported a decreased prevalence of hay fever and allergic rhinoconjunctivitis among farm children as compared to non farm children. In some studies a protective effect was also found for childhood asthma.

In the cross-sectional PARSIFAL study, the risk factors for allergy were studied in school-age children from rural environments. The results suggested that maternal exposure to a farming environment in pregnancy might protect against atopic sensitization and result in an upregulation of receptors of the innate immune system [3]. However, this protective effect might have been explained by a better memory of mothers of allergic children than mothers of healthy children. Only a birth cohort study with assessment of maternal activities and influential factors in pregnancy, could answer this question. The PASTURE study was designed. The findings confirmed the assumption: Allergic sensitization starts in utero under the influence of the maternal immune system and environmental determinants. In the cord blood of newborn babies immune responses differed by maternal exposures, in particular to animal stables during pregnancy. Children from farming mothers with exposure to animal sheds were significantly less sensitized to birch-, grass- or hazel pollen [4]. Allergen - specific IgE- antibodies in cord blood correlated with a lowered production of fetal IFN- $\gamma$ , a cytokine, which has been associated with allergy protection [5]. Maternal exposure to farm activities and to farm dairy products during pregnancy furthermore modulated cytokine production patterns in the offspring at birth. Significantly higher levels of IFN-  $\gamma$  and TNF- $\alpha$  were found in farm compared to non-farm children [6]. Induction of tolerogenic immune responses seems to occur already prenatally. Newborns of mothers who had taken vitamin D supplement in pregnancy had higher ILT3 and ILT4 gene expression, which is a marker of cells inducing tolerance [7].

It is not yet understood how these changes in immune responses relate to the protection from allergic diseases later in life. The further follow-up of the PASTURE cohort will investigate the role of early life immune response for allergy development until school age.

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**More Information:**

[www.efraim-online.com](http://www.efraim-online.com)

Contact:

EFRAIM Project Office

Nicola Korherr  
University of Munich Children's Hospital  
AG Prof. v. Mutius  
Lindwurmstr. 4  
D - 80337 München

e-Mail: Nicola.Korherr@med.uni-muenchen.de  
Phone: +49 (0)89 - 51 60 - 77 36  
Fax: +49 (0)89 - 51 60 - 44 52