

FORALLVENT - Final Activity Report

The prevalence of asthma and allergies across Europe is very high. About one in ten children suffers from asthma, a fourth of the population is affected by hay fever and about a third reveals a positive allergy test. Numbers for adults are even higher when work place exposures are also taken into account. The associated societal burden of costs for the treatment and management of these conditions is enormous. There is urgent need in health care for novel and innovative approaches to find avenues for the prevention and cure of allergic diseases. Current therapies are effective in controlling symptoms in most patients, but no curative or preventive approach has been found.

In the last decades there has been an accumulation of data not only on the epidemiological aspects of allergic diseases but also on specific risk and protective factors that govern the differential development of allergic diseases in rural and urban areas of Europe. Epidemiological observations suggest that strong protective factors are found in populations with a rural life style and with contact to farm animals. Children raised in these environments have lower rates of sensitization to environmental allergens than children growing up in rural environments but without exposure to farm characteristics. If it were possible to understand the precise nature of these ‘farm factors’ and the underlying immunological mechanisms, then we may be able to exploit the findings for preventive and therapeutic interventions.

The overall aim of the FORALLVENT project had been to create a platform and forum for the development of strategies enabling to close the gap between the science on allergic diseases and the practical application of its results in prevention, clinical treatment and the formulation of specialized foods.

Five specific aims of the project had been set. The project intended to provide a synoptic review of the relation between environmental factors related to microbial exposures assessed in previous experimental and epidemiological research within Europe and to identify the most relevant protective exposures. Such a synopsis has been written (“Comprehensive review of existing findings”) and can be downloaded on the FORALLVENT website. Another focus of FORALLVENT had been to foster interdisciplinary exchange between epidemiologists, allergologists, clinicians, biochemists, milk hygienists, dairy engineers and technologists, pharmacologists and other disciplines to generate novel avenues into the development of

protective and safe avenues for the prevention of asthma and allergies. Collaboration with SMEs and industry should be sought focussing on the protective effect of farm milk and other milk preparations. Furthermore, the follow up of the PASTURE birth cohort up to age 6 years had been envisaged to provide a human in vivo model to prospectively study the relevance of these factors. Finally the FORALLVENT project aimed at disseminating the collected information to the general public by means of professional media work (media conference and agenda setting in targeted medias) as well as to potential partners in industry, trade associations, interest groups, patient organisations, policy making bodies by means of target-oriented publications, fact sheets and the website.

To meet these goals researchers from the relevant disciplines were brought together to discuss and share ideas and knowledge, and to identify the gaps between the science and the applicability of existing findings to the prevention and treatment of allergic diseases. The FORALLVENT consortium consisted of 11 renowned partners from 8 European countries with expertise in pediatric allergology and pediatric and adult pulmonology (Children's Hospital of the Ludwig-Maximilians-University Munich, Germany; Children's Hospital in Schwarzach/Salzburg, Austria; Wroclaw Medical University, Poland; Department of Chest Diseases and Occupational Medicine at the University of Besançon, France), in environmental epidemiology (Institute of Social and Preventive Medicine of the University of Basel, Switzerland; National Institute for Health and Welfare in Kuopio, Finland; Institute of Epidemiology of Ulm University, Germany), in milk science (University of Natural Resources and Applied Life Sciences, Vienna, Austria) in dairy technology (Moorepark Food Research Centre, Cork, Ireland) and in communication (Ecos Communication, Basel, Switzerland). All contractors had experience in international research programs.

As a first step in the FORALLVENT project, a comprehensive review on the literature discussing protective environmental exposures related to the hygiene hypothesis was compiled (D1). Furthermore, a synoptic report highlighting the gaps in existing knowledge (D2) was written. In this second report, particular attention was given to identify gaps in existing knowledge and to propose future directions of work. Both reports are posted on the FORALLVENT website at <http://www.forallvent.info>.

In April 2007 the first interdisciplinary symposium "Environmental, genetic and immunological determinants of allergic and autoimmune diseases" was held in Basel,

Switzerland. There is evidence to suggest that the 'hygiene hypothesis' may not only apply to allergic illnesses but also to autoimmune disorders. Therefore, world experts in the field of allergy and autoimmune diseases such as diabetes type 1, rheumatoid arthritis, multiple sclerosis and inflammatory bowel diseases were brought together to discuss similarities and differences in the application of the 'hygiene hypothesis' in their respective fields of research. The summary of the meeting is posted on the FORALLVENT website.

The first immunology workshop in February 2008 summarized the current state of knowledge about the immune system and its function in the development of allergic conditions. The presentations and discussions from this workshop were summarized in a report (D3). One session of this workshop discussed recent findings from experimental studies. Inhalation of stable dust extracts by mice during sensitisation to ovalbumin was shown to inhibit the development of airway hyperresponsiveness and airway eosinophilia upon challenge. Stable dust extracts also redirected human dendritic cells away from Th2 immune responses. These findings support the notion that stable dust from animal farms contains strong immuno stimulating substances. Further discussions during this workshop related to the interplay of cells and mediators of the innate and adaptive immune response, including dendritic cells and regulatory T cells; the importance of gene-by-environment-interactions and gene expression studies as markers of cell activation and signal transduction; and the mechanisms underlying the protective effect of consumption of raw cow's milk or raw milk cheese in the gastrointestinal mucosa.

The second workshop was held in January 2009 at the Leiden University Medical Center, Netherlands. The focus of the workshop was to link epidemiological findings with potentially underlying mechanisms and to discuss the many emerging gaps in knowledge. The first session related to gut immunology. How are signals from the gut lumen transcribed into systemic immune responses controlling inflammatory reactions of other organs? The fact that despite a rich microflora with potentially strong inflammatory molecules, the gut is not in a constant state of inflammation indicates that strong anti inflammatory responses are at place guarding the integrity of the mucosa. The question whether a change in gut microflora can lead to the disruption of local tolerance must be addressed. The notion that food ingredients, i.e. vitamin A metabolites may play an important role in the balance between pro and anti inflammatory immune responses was discussed. By identifying and applying specific nutritional compounds, for example those in 'farm milk', the production of 'healthy foods'

induce tolerance may be possible. A considerable number of industrial companies had been invited. Many of them declared great interest in the subject and in future collaboration, although most of them were unable to attend the workshop. A resulting position paper discussing the potential avenues into innovative prevention strategies was delivered (D4).

Particular attention was paid to the attempt to develop an allergy protective milk formula. Results from previous studies indicate that the protective effect of farm milk consumption is stronger when the milk has not undergone heat treatment and remains unskimmed. Raw milk consumption undoubtedly bears risks, as raw milk is a potential substrate for almost any pathogen and has consistently caused outbreaks and fatal infections. The question how to produce a safe and at the same time allergy and asthma protective milk was discussed in two milk workshops. The first milk workshop in March 2008 at the Representation of the Free State of Bavaria to the European Union in Brussels, Belgium was entitled “Safe, protective, non-pasteurized milk”. The follow up milk workshop “Protective effect of farm milk consumption on the development of asthma and allergy” took place in September 2008 at the University of Natural Resources and Applied Life Sciences in Vienna, Austria.

Experts from the fields of allergology, immunology, epidemiology, food science, food technology, nutrition and communication met to exchange background knowledge and to discuss the potential for an intervention study to assess the allergy preventive effect of ‘farm milk’. The application of milk in an intervention study requires above all a safe product. The possibility of producing a safe unpasteurized cow’s milk was explored from all perspectives. The fact that the present laboratory technologies do not allow bacterial analyses of raw milk in a timely manner to discard contaminated samples and that dormant, damaged or non-viable microbes cannot be detected, prohibits the use of unpasteurized milk for study purposes.

Therefore, the alternative approach is to identify the compounds of raw cow’s milk which are responsible for the immuno-modulating, and thus, allergy-preventive effect. In this context, the microbial load of milk including viable, dormant and uncultivable micro-organisms or bacteria with probiotic properties have to be considered, as well as ingredient molecules such as fatty acids (e.g. conjugated linoleic acid-CLA) or proteins and peptides (such as whey proteins, e.g. immunoglobulins, β -lactoglobulin, α -lactalbumin and bovine serum albumin, and also lactoferrin). Originally, it was planned to invite partners from industry to both milk workshops. For confidentiality reasons the FROALLVENT Steering Committee discouraged

participation at this stage of the project. Involvement of profit orientated partners will occur when more precise strategies can be presented. A summary of the first milk workshop was recorded by partner 8 (D5) as well as a protocol for subsequent work (D6). After the second milk workshop partner 8 authored a position paper on potential avenues for the development of allergy protective milk formulas (D7), which is available on the FORALLVENT website.

The European Commission has committed significant funding for the conduct of the PASTURE study, a birth cohort in populations highly exposed to a diversity of microbial compounds in the environment, namely a farming environment. The funding period extended to the first birthday of the study child up to which the study objectives relating to the maturation of the immune response in the first year of life under conditions of heightened exposure were met. However, the identification of the protective exposures preventing the onset of asthma, hay fever and atopic dermatitis cannot be identified, because in many children the incidence of these allergic conditions occurs after the first year of life until the 6th year of life.

The FORALLVENT project provided resources for the follow up of the PASTURE cohort beyond age 1. Losses to follow up have been minimised through regular contact to the study participants. Information about activities of the study centres was provided for the participants by locally developed newsletters in each country and through local websites. The health status and the environmental exposure of the study participants have been assessed by parental interviews in yearly intervals in all study centres (Germany, Austria, Switzerland, Finland and France). The follow up questionnaires at age 3, 4 and 5 were drafted as part of the FORALLVENT project (D8, D9 and D10). As the recruitment period of the cohort expanded over 2.5 years, the participants are not all of the same age. Therefore, while the interviews at age 3 have been finalized, questionnaire assessment at age 4 and 5 is still ongoing and 4.5 year old children are being appointed for clinical examination. Results of the blood tests were sent to the participating families. A full clinical assessment will be necessary at age 6 years to assess the development of asthma and atopy among study subjects with sufficient certainty. Detailed standard operating procedure protocols (SOPs) for the clinical evaluation of asthma and allergies including allergy testing, lung function testing, skin examination and blood sampling for genomic, proteomic and peptidomic analyses have been prepared (D11, D12, D13 and D14). A field worker training for all centres was carried out in Bern, Switzerland to

assure standardized application of the procedures for the clinical assessment of the PASTURE children at age 6 across study centres.

Elaborate SOPs for sampling, storage and analyses of environmental dust samples and milk samples in the context of the PASTURE follow up study were set up to allow the identification of recently discovered novel immuno-modulatory substances (D17 and D18). The preparation for environmental sampling has started with a thorough review and synthesis of the existing knowledge from progress achieved in the analyses of dust and milk samples in ongoing national (France, Germany, Switzerland) and international (PASTURE, GABRIEL) studies and the GA²LEN Network of Excellence. From extensive literature review and use of own results a report on levels and composition of various compounds measured in house dust together with discussion of the most promising analytical techniques for house dust has been prepared. This report is part of Deliverable 16. Several FORALLVENT telephone conferences were held and a workshop was organized in Munich in July 2008 to discuss how to obtain optimal sample quality and the best analytic methods for environmental assessment. A report about the results of this workshop was delivered (D16). The cooperation among all partners involved in preparing the clinical assessment at age 6 years has been excellent, with all documents, questionnaires and material ready on schedule.

The FORALLVENT project ended in a final Symposium in January 2009 entitled "The Hygiene Hypothesis – New Insights into Allergic and Autoimmune Diseases". Top scientist from all over Europe came together in the magnificent Leopoldinum Hall of the University of Wroclaw, Poland to present newest insights on the role of the "Hygiene Hypothesis" in the discussion about the epidemiology of allergic and autoimmune diseases in Europe and the Western World. Representatives from industry, physicians and scientists from all parts of Europe and media representatives mainly from Poland took great interest in the event. Poland was chosen to host the Symposium as a bridge between Eastern and Western Europe. Abstracts of the presentations are available on the FORALLVENT website. After the Symposium several articles were published in public and professional newspapers and magazines in Poland and several TV interviews were broadcasted on national and local polish TV stations.

Ecos communication had been enlisted as partner in charge for professional dissemination of achievements in the FORALLVENT project to the broad public, to the scientific community,

to potential industrial partners and to patient organizations. All FORALLENENT events were published on the CORDIS website. The project website <http://www.forallvent.info> has become the main communication channel. It has regularly been updated. The website will remain active for at least 5 years after the end of the project. It gives information about the FORALLVENT partners and links to related websites. All workshop and conference results – as far as not confidential – have been published on the project website. It has been created to be informative to professionals, but also to the public. This strategy proved to be successful. Several other websites are referring now to pages published on www.forallvent.info. At the end of the project a handy standard brochure in English was produced, which addresses to opinion leaders, decision makers, scientists and the interested public. It covers the main topics and questions of the project and lists of addresses to obtain further information. It can be downloaded from the FORALLVENT website.

A highly motivated and extraordinarily cooperative group of outstanding European experts in various allergy and immunology related fields formed over the duration of the FORALLVENT project. This group includes not only partners in FORALLVENT and partners in the PASTURE cohort study, but also opinion leaders from related scientific groups, from related industrial branches and from the media, who have been inspired by the FORALLVENT idea.