PreDicta

# Post infectious immune-reprogramming and its association with persistance and chronicity of respiratory allergic diseases

## Newsletter N°5 May 2014

## *State-of-the-art in PreDicta*

CONTENT




*Predicta has received funding from the European Union's Seventh Framework Programme for research, technological development and demonstration under grant agreement No 260895*

## *Message from the Coordinator*

**Nikos Papadopoulos, Scientific Coordinator of PreDicta,**
*President of the European Academy of Allergy and Clinical Immunology (EAACI) .*

*« Dear Friends, with more than half of the project time behind us, we are now full speed toward the peak of PreDicta! Indeed, we are seeing great progress in all workpackages, as well as some of the ambitious targets we have set in the beginning, being at hand's reach! Our cohorts, pediatric and adult have been recruited and their initial results are currently being analysed. We have set up the complex and demanding methodologies required for some of our measurements, such as the lipid mediators of inflammation resolution. Finally our 'translational' end products, the virus chip and therapeutic DNAZymes, are making major progress. We are therefore happy with the developments and eager to reach and surpass the aims we have targeted. At the same time, it is now time to start thinking about the next steps, possible 'spin-offs' and opportunities that may arise from the materials and information that is been gathered in the context of the project, in addition to the described work. In all, developments remain exciting within PreDicta! We are looking forward to catching up once again during the EAACI Congress in Copenhagen and plan our next meeting in the fall ».*


**Predicta in numbers:**

- *Starting date:1/10/2010*
- *Duration: 5 years*
- *13 partners*
- *9 countries*
- *1 SME*
- *11 leading clinical and research centers*
- *6 000 000 provided by the European Commission*

## Longitudinal Cohort

The aim of the study gathering 5 centers across major cultural and climatic regions of Europe (Athens-Greece, Turku -Finland, Erlangen-Germany, Lodz-Poland, Ghent-Belgium) is to establish a cohort of preschool aged asthmatic children in order to prospectively follow them up for two years to evaluate changes in inflammatory responses and potentially associate them with respiratory infections and asthma persistence.

In Erlangen for instance, recruitments of the cohorts was reached by:

- placing a range of advertisements in the local newspaper and in a free magazine (Frankenkids)
- inviting parents to information evenings
- designing a flyer for distribution purposes
- contacting general practitioners, kindergartens and schools
- taking part at the "Day of Immunology" and presenting a poster with information about the PreDicta study
- making an appeal on the local network of the University Hospital of Erlangen

Recruitment of asthmatic children and matched controls was completed by the end of September 2013.

In total, **169 patients and 63 controls** have been included. Detailed data in respect to medical history, lung function, and bronchial inflammation (exhaled NO) and allergy evaluation (skin prick tests) have been recorded in all subjects. Material was obtained for genetic, microbiological, immunological, inflammatory and antibody assessments and have already started to be analyzed by different partners. All laboratory procedures have been standardized and performed with partners with no major issues.

All asthmatic children have been provided with and trained to fill in electronic diary cards and perform peak flow measurements, using a telemedicine equipment set, consisting of a cell phone implemented with an e-diary and a matched peak flow meter. The system was set to transmit the data via Wi-Fi in a specific e-platform. Moreover, all data collected will be inserted into an online database.

A significant number of children during symptomatic visits (i.e.; colds and/or asthma exacerbations) have been evaluated in all centers and are still being monitored.

Intense efforts (by emails, telephones, or post according to each center's availability) are made to maintain efficient follow up of the cohort. Some children have already completed their 2 years observation period.

Data have started to be analyzed and abstracts with baseline data from the pediatric cohort have been and will be presented in the annual EAACI congress (Milan 2013, Copenhagen 2014).

Developments in innate immune responses and asthma, active mechanisms of inflammation resolution from BFRAA and NKUA lab members have been presented in a workshop that took place in Porto Xeli, Greece on Friday 6th May 2014.

*Professor Marek Kowalski and his team from Lodz, Poland, with the first child recruited in the study*

***"We are glad and thankful to all scientists, parents and especially children that helped us to set up and put forward our studies."***

## Epithelial Models

Research at Imperial College comprises two Predicta work packages. The *epithelial models* work package is examining the reported deficiency in the ability of the cells lining the airway (epithelial cells) to produce critical antiviral molecules called interferons and also trying to identify molecules made by rhinovirus infected epithelial cells which have the ability to stimulate type 2 T lymphocytes, a cell type which is a key driver of allergic asthmatic disease.

To date Imperial and the other PreDicta sites involved have established the techniques and developed the reagents required to address these aims, and intensive studies are ongoing. Imperial has recruited and carried out studies on 14 patients. Notably, work on type 2 T lymphocyte stimulation has already highlighted some exciting new potential disease mechanisms which we hope could become targets for rhinovirus-induced asthma exacerbation therapies in the future.

A measure of the great success enjoyed by PreDicta funded research at Imperial College is the eleven directly Predicta funded research articles Imperial researchers have published in peer reviewed international journals, not to mention a similar number of related publications by Imperial's PreDicta investigators.

## Mouse Models

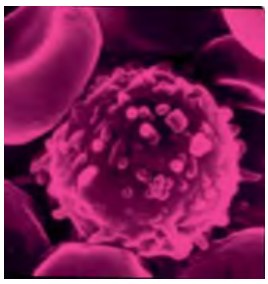
In the mouse model work package the team at Imperial have successfully developed a new model of rhinovirus-induced exacerbation of asthma driven by the clinically relevant allergen house dust mite and also, key to PreDicta's aims, a repeated rhinovirus infection model which recreates the repeated infections that everyone suffers throughout life. These new models and the use of established models have to date provided important insight into antibody responses to rhinoviruses, the disease implications of the aforementioned interferon deficiency in asthma and on the role played by T lymphocytes in virus control and disease.

## Virus Bacteria Interactions

The airway mucosal surface is a common site of colonization of many viruses and bacteria. Bacteria may colonize the human body without causing disease. In particular, Staphylococcus Aureus (SA) has been shown to colonize the airways in a great proportion of people without symptoms of disease (carriers). However, the attachment of a pathogen to mucosal surfaces is the first step towards respiratory disease.

We hypothesize that viral infections by Human Rhinovirus (HRV) may help SA to pass the epithelial barrier and gain access to the local immune system. Thus, although viral and bacterial microorganisms do have an impact on mucosal inflammation by themselves, the combination of both triggers may even cause more severe disease.

In order to understand the role of specific pathogens, pathogen subtypes and



interactions between microbial infections, we established an ex vivo human nasal mucosal explant model. Explants are ideal to mimic the in vivo situation as cells in explants maintain their morphology, normal cell-cell contacts and consequently, the three-dimensional structure of the tissue is retained. This contrasts to nasal epithelial cell culture systems, where such structure is lost.

Mucosal explants from healthy human nasal inferior turbinates, turbinate mucosa of allergic rhinitis patients, and mucosa derived from Chronic Rhinosinusitis with Nasal Polyps (CRSwNP) were infected with HRV and/or SA.

We observed that HRV shows a faster invasion through CRSwNP epithelium compared to control turbinates, which can be explained by the decreased expression of epithelial tight junction molecules and the impaired interferon response in CRSwNP explants after HRV exposure.

These findings implicate that there is an inadequate response to the viral attach in CRSwNP patients, which is confirmed by observations that CRSwNP patients often have longer lasting and more severe common cold symptoms compared to healthy controls. Furthermore, HRV infection facilitates the invasion of SA into the nasal mucosa and nasal polyp tissues, which then gives rise to a whole series of immune events.

## Diagnostic

Since the beginning of PreDicta, the team based at the Medical University of Vienna (*Photo*) has been working hard to develop the first serological test for the identification of the culprit of rhinovirus (RV) strains involved in exacerbations of asthma and other chronic inflammatory diseases of the lung.

Since October 2010, the group has produced altogether 40 recombinant proteins and protein fragments from different RV strains representing all three RV groups, RV-A, RV-B and RV-C. Furthermore, in close collaboration with Biomay AG, they established pilot immunoassays for measuring rhinovirus specific antibodies, which were further used to investigate mechanisms of memory immune responses during RV infections.

These findings published in the FASEB Journal (Niespodziana *et al.*, FASEB J. 2012 Mar; 26(3):1001-8) have received high attention in both the scientific community and the lay public and were even commented by the prestigious Journal of Allergy and Clinical Immunology in February 2012.

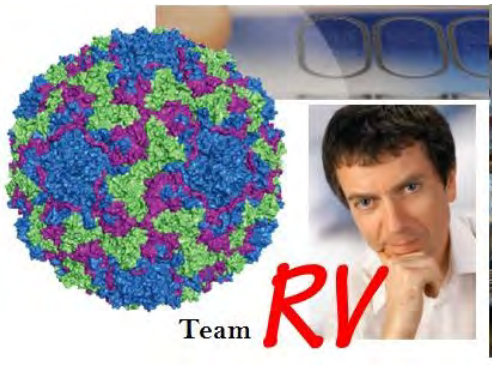
This was mainly due to the fact that they provided evidence for novel mechanisms of how rhinoviruses may escape the control of the human immune system.

The group reported that the immune response against rhinovirus was directed against an N-terminal peptide in the VP1 protein, which, paradoxically, is located inside the viral capsid and suggested that the misdirection of the antibody responses against non-neutralizing epitope may serve as a possible explanation for the lack of protection against recurrent RV infections.

In January 2014, the group produced their first prototype RV micro-array, which uses significantly low serum volumes, is highly sensitive and allows simultaneous detection of different antibody classes to a broad spectrum of viral antigens.

The currently used PCR-based methods demonstrate the presence of the rhinovirus in the respiratory tract by a direct detection of the viral RNA, however, they cannot confirm whether an infection with certain RV strains took place and whether the immune system has responded.

Should this chip-based serological test prove indeed to be effective in identifying the most common and clinically relevant rhinovirus strains involved in respiratory diseases, it will be a very useful basis for the development of novel therapeutic or prophylactic intervention strategies.




***Medical University of Vienna, from the left*:** Rudolf Valenta, Daniela Gallerano, Katarzyna Niespodziana and Clarissa Cabauatan

## Tonsil studies

Triggering of specific Toll-like receptors and proinflammatory cytokines breaks allergen-specific T-cell tolerance in human tonsils and peripheral blood

The generation and maintenance of allergen-specific T-cell tolerance is a key step in healthy immune responses to allergens and successful allergen-specific immunotherapy. Breaking of peripheral T-cell tolerance to allergens can lead to the development of allergies, but the mechanisms are not completely understood. We sought to identify molecular mechanisms that break allergen-specific T-cell tolerance in human subjects.

We showed that certain innate immune response signals and proinflammatory cytokines break allergen-specific CD4+ T -cell tolerance in normally unresponsive subjects, which might lead to the development or exacerbation of allergic diseases after encountering microbes or inflammatory conditions.

*PreDicta partners involved: SFI (Switzerland) and Turku University Hospital (Finland)*

Distinct regulation of tonsillar immune response in virus infection

Exacerbations of childhood asthma, adult asthma and chronic obstructive pulmonary disease have been closely linked to positive virus findings. Pediatric studies have shown a strong link between susceptibility to rhinovirus-associated wheezing and the development of asthma.

The mechanisms of these associations are incompletely known. Considering tonsils as organs that have a role in immune tolerance induction, we investigated the associations between in vivo intratonsillar T cell cytokine responses and viral infections in palatine tonsil samples obtained from 143 elective tonsillectomy patients of which one half reported allergy.

Our results showed that tonsillar cytokine expression is closely related to existing viral infections, age, and allergic illnesses and shows distinct clusters between antiviral and immune regulatory genes.

This study provides several new and potentially important findings considering tonsils as a new *in vivo* model for the understanding of immune response development and immune tolerance induction.

*PreDicta partners involved: SFI (Switzerland), Turku University Hospital and University of Turku (Finland)*

**References:**


Kücüksezer UC et al. Triggering of specific Toll-like receptors and proinflammatory cytokines breaks allergen-specific T-cell tolerance in human tonsils and peripheral blood.J Allergy Clin Immunol. 2013 Mar;131(3):875-85.

Jartti T et al. Distinct regulation of tonsillar immune response in virus infection. Allergy. 2014 May;69(5):658-67. doi: 10.1111/all.12396. Epub 2014 Mar 29


## The PreDicta Consortium

*Predicta Consortium meeting Vienna, January 2013*

## The protein BATF contributes to asthma development

Many people, especially children suffer from allergic asthma. To date it is impossible to cure the disease and only the symptoms like e. g. shortness of breath and wheezing can be alleviated. In order to develop novel therapeutic strategies and preventative actions, it is necessary to examine the underlying mechanisms of the disease.

*"The important role of BATF in Th2 and Th17 cells, as well as in B cells makes it an interesting target for asthma research".*

In a recently published study, researchers from the Department of Molecular Pneumology, University of Erlangen-Nürnberg (Germany) were able to show that the protein BATF (basic leucine-zipper transcription factor, ATF-like) plays a major role in asthma. BATF has already been described to be essential for the differentiation of Th17 cells and also contributes to the development of IgE antibodies.

In collaboration with the Children's Hospital in Erlangen and PreDicta Work-Package 1 teams, we analyzed blood samples of pre-school children with and without asthma. We observed an increase in BATF expression in children with asthma, which were not treated with corticosteroids and came to the hospital for a symptomatic visit. Furthermore, in an experimental model where BATF was missing, we showed that symptoms of asthma, e. g. lung inflammation, airway hyperresponsiveness or mucus production were markedly decreased.

Taken together these results indicate that BATF is important for the pathogenesis of asthma. These findings may help to improve current asthma therapy, to establish novel therapeutic strategies or even to prevent asthma development.

**Reference:**

Übel C et al. The activating protein 1 transcription factor basic leucine zipper transcription factor, ATF-like (BATF), regulates lymphocyte- and mast cell-driven immune responses in the setting of allergic asthma. J Allergy Clin Immunol 2014; 133:198-206 e9.

## The factors IL-6 and T-bet regulate allergic asthma in opposed fashion

Asthma is a chronic inflammatory disease of the airways. Some patients suffer from life-threatening asthma attacks including breathlessness, increased mucus production and obstruction of the bronchi. The protein T-bet (T box expressed in T cells) acts as a molecular switch that supports the development of specialized immune cells with anti-inflammatory properties and plays a protective role in asthma. In contrast, the messenger substance interleukin 6 (IL-6) favors the differentiation of immune cell subsets, which provoke asthma exacerbations.

In the course of the PreDicta study and in collaboration with the Children's Hospital in Erlangen, Germany, the University of Erlangen-Nürnberg team analyzed peripheral blood mononuclear cells (PBMCs) from pre-school children with and without asthma. A negative correlation between the expression of IL-6 and T-bet was observed: children with asthma and low T-bet expression exhibit high levels of IL-6.

In an experimental model of Subcutaneous ImmunoTherapy (SIT) for asthma, reduced levels of IL-6 were found.
In another experimental setting of asthma, antibodies were used to block the signaling pathway by blocking IL-6 receptor alpha chain (IL-6Ralpha). This treatment resulted in ameliorated airway inflammation, accompanied by reduced T helper 2 (Th2) and Th17 cells, which are known to play an important role in the development of asthma.

*"Taken together, these results demonstrate that both SIT and the use of antiIL-6R antibodies results in amelioration of the allergic response".*

*This work was supported by the Department of Molecular Pneumology (University Hospital Erlangen), SFB grant 643 (B12), the Children's Hospital Erlangen and the European study PreDicta.*

**Reference:**

Koch, S et al. IL-6 activated integrated BATF/IRF4 functions in lymphocytes are T-bet-independent and reversed by subcutaneous immunotherapy. *Sci. Rep.* 2013; 3.

## Specific IgE to *Staphylococcus aureus* enterotoxins (SE-IgE) is associated with asthma, specifically severe asthma

Years of research in the URL lab of the University of Ghent, Belgium have demonstrated that SE-IgE antibodies in the mucosal tissue of chronic rhinosinusitis with nasal polyps are associated with asthma comorbidity. SE-IgE serves as a tissue marker of a severe Th2-biased immune reaction, and indicates the impact of *S. aureus* enterotoxins, also called superantigens, on the local immune system. It was a logic next step to ask the same question for severe asthma: is SE-IgE, this time measured in the serum, associated with severe disease?

Specific IgE antibody concentrations against enterotoxins, grass pollen and house dust mite allergens and total IgE levels were measured in adult cohorts of 69 control subjects, 152 patients with non-severe asthma, and 166 patients with severe asthma. Logistic regression analyses demonstrated significantly increased risks for enterotoxin IgE-positive subjects to have any asthma or severe asthma versus enterotoxin IgE-negative subjects. Of interest, 21% of patients with severe asthma with enterotoxin IgE were considered non-atopic, possibly explaining "intrinsic" asthma. Increased oral steroid use and hospitalizations in the last 12 months, and a lower FEV1 were significantly associated with enterotoxin IgE and non-atopic asthma.

In a large European study using the GALEN network, we were then able to confirm that SE-IgE was associated with asthma in the general population in a concentration-dependent manner, independent of atopy.

Just recently, these results were also confirmed in Korea: SE-IgE was independently associated with adult-onset asthma in adult community populations, and again, smoking increased the risk of being SE-IgE positive. SE-IgE was again identified as the major determinant factor for total IgE.

*"These findings support a role for S. aureus enterotoxins in severe airway disease and encourage further research".*

**References:**


Bachert C et al. Specific IgE against *Staphylococcus aureus* enterotoxins: an independent risk factor for asthma. J Allergy Clin Immuno. 2012;130:376-381

Tomassen P et al. Staphylococcus aureus enterotoxin specific IgE and its association with asthma in the general population: a GA²LEN. Allergy 2013;68:1289-97

Song WJ et al. Staphylococcal enterotoxin sensitization in a community-based population: a potential role in adult-onset asthma. CEA 2014;44: 553-562


## *178 Members of the European Parliament call for coordinated European action on allergies*

In October 2013 11 Members of the European Parliament (MEPs) proposed a draft Written Declaration on recognising the burden of allergic disease, calling upon the Commission and Member States to recognise the burden of allergic disease and develop policy tools to combat this illness. At the end of the consultation period, the Declaration was supported by a total of 178 MEPs.

The MEPs have made a number of recommendations to help mitigate the impact of allergic diseases, which affect over 150 million Europeans:

**EU Member States were called upon to implement national programmes, to provide better medical training and to encourage scientific research.**

More info: http://www.eaaci.org/activities/written-declaration-on-allergies.html

# Next Events


The European Academy of Allergy and Clinical Immunology cordially invites you to its 2014 Annual Congress, which takes place in Copenhagen from 7–11 June.

More info: http://www.eaaci2014.com/


The 2nd International Severe Asthma Forum will take place in Athens from the 13 to the 15 November 2014.
The general purpose of the meeting is to assemble dedicated people in the field, involving both experienced key opinion leaders and young scientists.

More info: http://www.eaaci-isaf.org/

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