PreDicta News

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

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Message from the Coordinator

Nikos Papadopoulos
National and Kapodistrian University of Athens

When it comes to scientific excellence, there is little doubt that the PreDicta group gets top marks. So no surprise there: the first high-impact publications are turning up, just at the time of our first report.

What I find more impressive however, is how interactions between the group members produce synergies; new ideas, extended collaborations, further activities wider than the project.

This was once again the experience in our spring meeting in Davos (March 2012). All the elements were there: meeting with friends, which is always great, reporting achievements, a fulfilling act as well, but particularly, revealing novelty, opening up new possibilities. And even more: this fertile environment made participants enthusiastic, with additional proposals of interaction coming up.

One of these ideas, which we will most certainly pursue, is the possibility of a PreDicta Summer School. Most of us had great experiences with Summer Schools: really memorable events of the highest educational value and plenty of time for social interaction. On the other hand, when we leave our usual 2-day meetings, there is this impression that more time and interaction would have been very welcome. For this we will have to find additional funding. It comes naturally, as there are so many possible 'extensions' of the program already in mind that we'll have to do that soon.

Writing these lines, I'm also about to compile the information for the Report to the Commission. With all work-packages progressing smoothly and even the small problems that had occurred already solved, I'm hopeful that this will be seamlessly done.

Some more good news: we have Dahlia back with us, and she is now accompanied by her beautiful daughter ! Congratulations! Since the beginning of PreDicta, we had (at least) 3 babies by members of the consortium; isn't this real creativity!

We also thank Beatrice for helping us keep on track for these last months. We hope to have the opportunity to collaborate with her again in the future.

The group will have the opportunity to meet in Geneva, as we did last year, during the EAACI Congress. A good time to catch-up and, among other, plan our next face-to-face meeting in autumn.

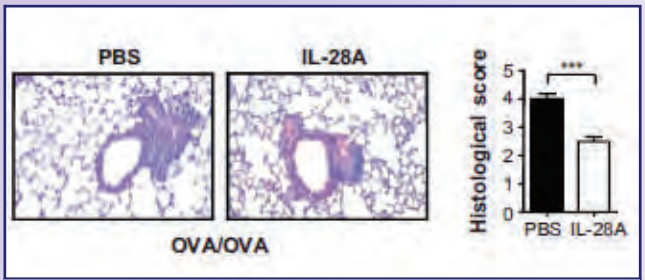
PreDicta achievements during the first period (Dec 2010-May 2012)

During its first 18 months the PreDicta Consortium was able to setup and standardize all the necessary models and methodologies required to achieve its various objectives. These models have already started producing answers to substantiate our hypothesis: three papers have been published and another four submitted during this period.

A **detailed protocol** for a longitudinal cohort of preschool children has been established and approved by the Institutional Ethical Committees of all partners involved. Relevant laboratory procedures were optimized and standardized among partners. Subsequently, inclusion of subjects, sampling and follow up of patients has started to take place. Until the completion of the first period, 70 patients and 12

controls have been included. Patients have adhered to the protocol and attended the planned visits; a number of symptomatic visits have also taken place.

We developed a mouse model of repeated RV infections using minor group RV1B and RV29. Strong cross-serotype RV-specific IgG responses in serum and bronchoalveolar lavage were induced towards the RV capsid protein VP1. IgA responses were

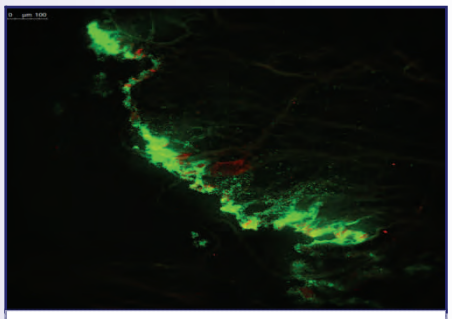


Treatment with IL28 suppresses Th2/Th17 cytokine production and ameliorates allergic airway disease in a mouse model. **Koltsida et al. EMBO Mol Med 2011;3:349**

weaker, requiring two infections to generate detectable RV-specific binding. Similarly two or more RV infections were necessary to induce neutralising antibodies. Immunization strategies produced stronger cross-serotype neutralizing IgG responses. This model, unlike studies in humans, allowed the understanding of RV-neutralizing antibody generation *in vivo* and will facilitate antibody-mediated vaccine design and development. Additional mouse models, using allergen stimuli or poly I:C stimuli are also in development. These models will help further evaluate our hypothesis, but also investigate mechanisms and potential interventions.

Protocols for the culture of bronchial and nasal epithelial cells have been optimized and comparisons done between laboratories. Viruses are grown centrally and shared across the groups. A considerable number of donors have been sampled and cells cultured and frozen. Ongoing experiments will show whether frozen cells behave as fresh ones and could be used instead of or interchangeably. Molecular techniques such as DNA DISH, ChIP have been optimized for use with primary epithelial cell infection with rhinovirus. Other techniques such as RNA-sequencing (RNA-seq) and culture of primary cells with Th2 cytokines, are currently being optimized.

An ex-vivo model of interactions between RV and Staphylococcus Aureus infections in human nasal mucosal models has been set up. Pro-inflammatory cytokines were clearly increased after RV and/or SA infection. Evaluation of RV16 and SA mucosal spread was performed by confocal microscopy. RV as a single infection led to very few infected cells of the outer epithelial barrier within 48h; S. Aureus as a single infection did not affect the epithelial integrity and only few bacteria attached to the epithelium. However, after infection with RV16 for 48h and S. Aureus for 24h, the whole epithelial barrier was heavily infected by RV, and S. Aureus was able to pass through the basement membrane and invade the mucosa. Furthermore, a clinical cohort designed to investigate the effect of upper airway infections on the natural course of allergic rhinitis and chronic sinusitis with nasal polyps, has been initiated.

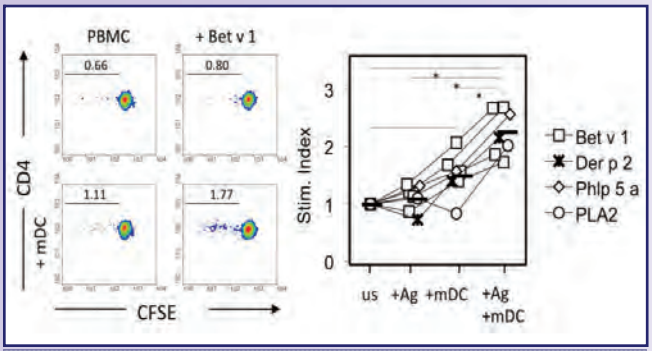


Combined exposure with RV16 and *St. aureus* compromises the epithelial layers and permits penetration of both microbes – Confocal image

Looking into the **effects of the innate immune response on tolerance,** two studies were performed. In the first study cellular and molecular mechanisms of oral tolerance induction to allergens in human tonsils was demonstrated. CD4+FOXP3+ Treg cells and pDCs constitute important T- and dendritic cell–compartments in tonsils. Tonsil pDCs have the ability to generate functional CD4+CD25+CD127-FOXP3+ Treg cells with suppressive property from naive T cells. CD4+FOXP3+ Treg cells proliferate and co-localize with pDCs in-vivo in T-cell areas of tonsils. Tonsil T cells did not proliferate to common allergens. Depletion of FOXP3+ Treg cells enables the allergen-induced proliferation of tonsil T cells, indicating an active role of Treg cells in allergen-specific T-cell unresponsiveness. A positive correlation between the percentages of FOXP3+ Treg cells and pDCs is observed in tonsils from non-atopic individuals.

In the second study we demonstrate that IL-32 is expressed in many cells, including natural killer cells, endothelial cells, and keratinocytes. The expression of IL-32 in NHBE cells after stimulation with TNF-a, IFN-g, or rhinovirus infection suggests that IL-32 plays an important role in the host defense against viral or bacterial pathogens. It was also demon-

strated that after IL-32 knockdown, VEGF secretion was upregulated, and tubular formation of HUVECs

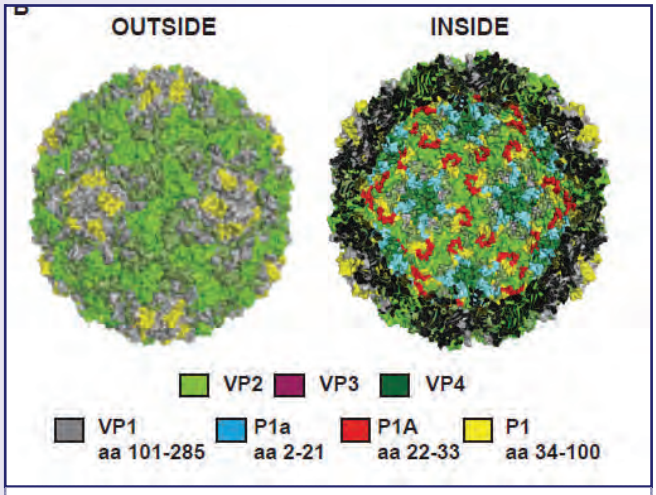


mDCs play a role in breaking allergen-specific T cell tolerance (submitted)

cultured with supernatants from IL-32 siRNA–transfected NHBE cells significantly increased, indicating that IL-32 is an important inhibitor of airway remodeling in asthmatic and IL-32 decreases VEGF production and angiogenesis. Detectable serum levels of IL-32 in asthmatic patients were a strong predictor for a positive response to asthma treatment.

In order to study the **active mechanisms of inflammation resolution**, we developed and optimized LC-MS/MS methodologies that enable qualitative and quantitative determination of selected specialized pro-resolving lipid mediators (SPMs). The SPMs included in our studies are representative molecules of the major families of bioactive lipid mediators of resolution: Lipoxin A4 (LXA4), Resolvin D1 (RvD1), Resolvin E1 (RvE1), Protectin D1 (PD1) and its stereoisomer 10S,17S-DiHDoHE (PDX). We have also expanded our panel towards pro-inflammatory molecules: Prostaglandin D2 (PGD2), Leukotriene B4 (LTB4) and Eoxin C4 (EXC4). We validated the analysis of these molecules in biological matrices of interest i.e. murine serum and lung as well as different culture media. Validation demonstrated the high sensitivity and selectivity of our approaches resulting in low limits of detection / quantification and ability to distinguish between stereoisomers by chromatographic separation. LC-MS/MS methodologies developed were applied in an established preclinical model of allergic airway inflammation induced by ovalbumin sensitization and challenge. The resulting lipidomic profiling data revealed that mediators of the protectin family (PD1, PDX) are the first to be generated in the early phase of the allergic response, while their levels increase as the response proceeds into the resolution phase.

By studying **antibody responses to RV**, the group reported that the major RV immune response was directed against an N-terminal non-neutralizing peptide in VP1, which, paradoxically, is located inside the viral capsid. We suggested that the misdirection of antibody responses may serve as a possible explanation for the lack of protection against recur-



Major antibody response directed against P1A, located inside the RV capsid. **Niespodziana K et al. FASEB J 2012;26:1001**

rent RV infections. These findings, which have received considerable attention by the scientific and lay press, may pave the road for the further development of diagnostic tests for rhinovirus infections and of rhinovirus vaccines which may help to prevent airway diseases such as asthma and COPD.

As an ambitious therapeutic strategy, we aimed to **develop and characterize DNAzymes** with the potential to prevent rhinovirus amplification and subsequently reduce rhinovirus-related morbidity of asthmatic patients. In parallel, development of novel suitable transfection methods ensures timely and improved transfection in-vivo. Putative target regions in the RV genome were identified and cloned into two different vectors. In the context of looking into the RV sequence, interesting observations in relation to RV evolution were made, showing strategies the virus is taking to avoid the human innate immune response. Cloning was performed using a T7 RNA polymerase allowing us to amplify viral target RNA and perform the cleavage assay. A broad set of DNAzymes was developed, either specific against conserved or unspecific against closely related sequences. So far no active DNAzymes could be found. In parallel, novel liposomal carriers were developed, optimized for size stability, and screened for in-vitro knock-down efficacy in cell culture.

PreDicta represented at the « Europe day » in Belgium

How is Europe related to my city or village? In fact, in many ways Europe is closer than you would think! That was what the province of East-Flanders (Belgium) wanted its inhabitants to find out.

Sunday 6th of May 2012, the province of East-Flanders organized a 'Europe day' for their inhabitants to learn about some European projects and to promote them. This promotion day consisted of a bicycle ride along several companies active in Europe. One of the stops was Sint-Gillis-Waas, there was an interactive projects market for the visitors to participate in.

Of course several departments of the University of Ghent participated in this promo day. One of which was the Upper Airways Research Laboratory of Prof. Bachert, to present the PreDicta project. Visitors could undergo skin prick tests to determine whether or not the patient had some kind of allergy or get an idea about their lung function with a peak flow meter. The enthusiasm was big, and so Dr. Tineke Dutré and Dr. Frederic Acke tested more than 120 people during that afternoon. Some got confirmation on (not) having an allergy, some discovered to which allergen they reacted / from which allergy they had been suffering.

The Predicta project was explained, with the different work packages and the multitude of European co-operations between the centers. Moreover, the link between infection and allergy was new to most people. Testing their new insights, the spectators could answer some questions to win 'The allergy survival guide', (Prof. Ph. Gevaert) giving understandable information for children and adolescents concerning allergy and atopy.

PreDicta' series of portraits: Spotlight on PreDicta researchers

Nan Zhang
University of Ghent

What is your scientific background?

***E.N.T Specialist** in the Otolaryngology Department of West China Medical University Hospital, Chengdu, China.

***Ph.D. thesis** on the genetic research of hereditary hearing loss, especially on mitochondral DNA mutations in hearing loss.

***Assistant professor** in otolaryngology-head & neck surgery of Zhongshan City People's Hospital.

***Postdoctoral fellowship** at the Upper Airway Research Laboratory, University of Ghent.

Work fields: Immunology of upper airway disease, the microbiome and its interactions innate and adaptive immunity, interaction of viruses and bacteria in the initiation and persistence of the disease, international networking on airway disease

What are your lab's main discoveries related to respiratory allergic diseases/asthma?

We focus on the pheno- and endo-typing upper airway disease in order to understand the link to lower airway disease manifestations. In this respect we recently identified specific IgE against Staphylococcus aureus(SE IgE) enterotoxins as a maker of systemic airway disease in patients with nasal polyps. We then observed SE IgE an independent risk factor for asthma Europe wide and specifically associated with severe asthma. This discovery allows totally different understanding especially of non atopic severe asthma as a infectious disease driving by bacterial supperantigens.

What is your role in PreDicta?
I am working for WP4: Virus-Bacteria interactions, specially in developing ex-vivo nasal mucosal model for identifying viral and bacterial interactions on the epithelial barrier, characterizing innate as well as adaptive immune defense mechanisms. We just discovered deficits in the immune response to both viruses and bacteria. These are very interesting finding which help us to understand the persistent disease.

For you, what was the most valuable scientific discovery in 2011/2012?
Synthetic Genome brings new life to bacterium: Dr. Venter copied the DNA from one species of bacteria and inserted it into another. The second bacteria made all the proteins and organelles in the so-called "synthetic cell," by following the specifications implicit in the structure of the inserted DNA. "synthetic cell" is presenting the research as a landmark achievement that will open the way to creating useful microbes from scratch to make products like vaccines and biofuels.

For you, what was the best scientific article published in 2011/2012?
Late Interleukin-6 Escalates T Follicular Helper Cell Responses and Controls a Chronic Viral Infection. *James A. Harker, Gavin M. Lewis, Lauren Mack, and Elina I. Zuniga. Science 11 November 2011: 825-829.*

One book you would recommend?
Superantigens and Superallergens , Gianni Marone, KARGER 2007. This book focuses on my areas of interest.

What career were you planning while being a child?
As a child I was always dreaming to become a medical doctor and I actually worked as a E.N.T surgeon for 10 years in China, thus my dream came true. Since I came to Europe, I had the opportunity to start my career as a researcher, building up on the knowledge about the diseases I already gathered as a surgeon. To be a researcher is completely different thing, more challenging but also more rewarding once the paper has been accepted.

Have you participated in other EU projects?
Personally, PreDicta is my first EU project, no wonder as I came from Asia only 6 years ago. However the chair Professor Bachert already participated in the successful GALEN program and is also currently part of the MeDall project (www.medall-fp7.eu/).

What is your opinion about the PreDicta network?
The network is very active and communicative. I specially enjoy PreDicta meetings, which are important to keep us updated with the progress and facilitate the exchange of knowledge and opportunities.

Relevant clinical trials started in 2012 *(outside PreDicta)*

- A 6-month safety and benefit study of inhaled fluticasone propionate/ salmeterol combination versus inhaled fluticasone propionate in the treatment of 6,200 pediatric subjects 4-11 years old with persistent asthma. Sponsor: GlaxoSmithKline Research & Development (UK). Start date: 9/01/2012. More information here
- Pediatric patients age 6-12 yrs with uncontrolled mild persistent asthma. Sponsor: Technische Universität Dresden (DE). Start date: 15/03/2012. More info here
- Evaluation of any steroid sparing effect of beta blocker therapy on airway hyper-responsiveness in stable, mild to moderate, asthmatics. Sponsor: Tayside Clinical Trials Unit, University of Dundee (UK). Start date: 26/01/2012. More information here
- A 12-Week, Randomized, Placebo-Controlled, Dose-Ranging, Efficacy and Safety Study of Mometasone Furoate Metered Dose Inhaler in the Treatment of Children Ages 5 to 11 Years With Persistent Asthma. Sponsor: Schering-Plough Research Institute, a Division of Schering Corporation. Start date: 06/02/2012. More information here
- A multicenter, randomized, double-blind, placebo-controlled, parallel-group study to evaluate the efficacy and safety of intraseasonal specific short-term immunotherapy with depigmented glutaraldehyde polymerized birch pollen allergenic extracts (Depiquick® Birch) in patients with allergic rhinitis and/ or rhinoconjunctivitis with or without intermittent asthma. Sponsor: Novartis Pharma GmbH (DE). Start date: 29/02/2012. More information here