PreDicta News

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

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Content summary

Message from the coordinator

Time flies. Just as I was trying to find time to file PreDicta's launch photos, I realized that we're almost 6 months into the project! But then again, all workpackages have started their activities, some preparatory, some well into core business, as you will read in more detail below; we even have had a couple of papers and some abstracts submitted already!

The idea for this newsletter came from discussions on how we can keep everybody informed and aligned with all the activities of the project and keep in contact in-between our physical meetings. So our editorial team, headed by Dahlia, will gather the info, squeeze it into pleasantly readable bits and send it out to everyone every 3 months or so. With your help, of course, we will include news and activities from each workpackage, present profiles of PreDicta members, highlight each time one of the Teams and their work in and outside PreDicta. There will also be news on publications of interest to PreDicta, tips on how to use the PreDicta tools, such as the website, ads, if you send any, and information on our own publications and events. By the way, taking advantage of the EAACI Congress in Istanbul where many of us will participate, we arranged a catch-up meeting on Monday June 13, 17:00-19:00. Join us if you're around!

I truly hope that you'll enjoy reading this newsletter. It aspires to be an open communication medium for the PreDicta Community, so if you have any ideas (e.g. if you write poetry in your free time, or you're good with scientific jokes, or you like the picture of a particular gel from your last experiment) and want to share it with your PreDicta friends, don't hesitate to send it in!

Best regards from Athens. Hope to see you soon!

Nikos

What's new in PreDicta's Work packages?

Progress made in PreDicta work packages from month 1 to 4 (October 2010 to March 2011)

In WP1: Longitudinal cohort

The WP1 teams from all 5 centers have been assembled: Vicky Xepapadaki – Irena Roumpedaki- (Athens), Marek Kowalski – Anna Sobanska (Lodz), Theodor Zimmermann – Eva- Maria Ruth (Erlangen), Claus Bachert - Dutre Tineke (Ghent) and Jartti Tuomas- Heikki Lukkarinen (Turku) are the "pediatric teams" responsible for recruiting. The lab teams are Nikos Papadopoulos - Maria Passioti (Athens), Marek Kowalski - Anna Sobanska (Lodz), Susetta Finotto - Anna Graser - Sonja Trump (Erlangen), Claus Bachert - Nan Zhang (Ghent), Jartti Tuomas - Tytti Vuorinen (Turku). The detailed protocol has been drafted, discussed between the participants and finalized. The laboratory techniques for processing of fresh PBMC have been standardized. RV suspensions required for PBMC cultures are getting prepared and will be provided in time to all 5 centers All centers have now submitted, or are about to submit for Ethics approval. An open call for the electronic spirometers and diaries has been launched. As it is important to have a common approach between all centres 2 training sessions, one for the recruiting clinicians and one for the scientists performing the PBMC cultures will take place in parallel in Athens on 20-21st of May.

In WP2: Mouse models

Work has got off to a great start once Nathan (and most of us) recovered from his headache after the Athens visit! In the Imperial labs we have begun to characterise repeated infections with the minor group serotype viruses RV1B and RV29 in BALB/c

mice – finding as we did in the pilot experiments, that inflammation is magnified on the second infection relative to the first, whether sequential infection are with the same serotype, or with different serotypes.

Predicta's first mouse paper on antibody responses to human rhinovirus in a mouse model of infection has been submitted to the Journal of Immunology, but sadly also just rejected !

In addition we have begun investigating the induction of Th17 cells by RV infection. We have visited our colleagues in Erlangen and have agreed initially to investigate IL-17 deficient mice in the RV model, these mice are in the process of being transferred to Imperial for these studies.

We have applied for an EAACI Short term Fellowship to permit Aikaterini Chairakaki from Vangelis' group in Athens to visit Imperial for 2-3 months to learn how the mouse model works, and to teach us some immunology!

We are looking forward to making further progress (plus or minus a few headaches) over the next few months.

In WP3: Epithelial models

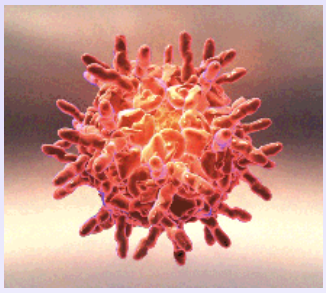
We have begun our recruitment of groups of 12-15 adult subjects for each of the following 4 groups:

- Non atopic normal subjects with no asthma
- Atopic subjects with no asthma
- Non atopic subjects with asthma
- Atopic subjects with asthma

Each subject will undergo clinical characterization for asthma (spirometry, non specific BHR) and atopy (skin prick tests), bronchoscopy and bronchial brushing to obtain primary bronchial epithelial cells which will be passaged in culture until expanded sufficiently to provide enough cells for experimental purposes – normally at passage 4. The production of type I and type III IFNs upon RV infection will be assessed in all four groups of patients and association between deficient IFN expression and atopy or asthma determined.
We are also analysing deficient IFN responses in primary bronchial epithelial cells from children with and without asthma, and in bronchoalveolar lavage cells in adults with and without asthma.

Heidi Makrinioti from Nikos' group in Athens is spending time in London setting up work investigating interactions between bronchial epithelial cells and T cells following on from the work she did previously with Nikos and Cezmi and Mubeccel Akdis in Davos. We are looking forward to exciting initial results from this work over the coming months.

In WP4: Virus-Bacteria interactions



Our objectives for the first 5 months of this project were:

1) to develop an ex-vivo mucosal model in which we can study the interactions of rhinovirus and Staphylococcus aureus infections. We have already established a model using Herpes simplex virus before, from which we could transfer a lot of technical details. We furthermore have transported rhinovirus 16 from Athens to Ghent, have learned the techniques in Nikos lab, and meanwhile have successfully propagated the rhinovirus. The first tests to infect the mucosal tissue by rhinovirus already have been performed, and we are in the process of localizing the virus in the tissue. Nan Zhang and Lara Derycke are performing the lab work.

2) to prepare all documents including the application for the ethical vote for the pediatric cohort study, and to discuss and plan the study with our pediatric colleagues. Tineke Dutre did all that clinical work, we are ready to go!

In WP5: Immune regulation

Our studies demonstrated that human palatine tonsils are allergen-specific immunotolerance inducing lymphoid tissues with high expression of CD4+ Forkhead box P3 (FOXP3)+ regulatory T (Treg) cells. Allergen-specific FOXP3+ Treg cells are identified in human tonsils using MHC class II peptide tetramers. Tonsil T cells show diminished proliferative responses to common allergens, which is abolished after depletion of FOXP3+ Treg cells. Direct analysis of biopsies and freshly isolated cells indicate that plasmacytoid DCs (pDCs) dominate in palatine tonsils and play a role in FOXP3+ Treg cell differentiation from naïve T cells. CD4+FOXP3+ Treg cells co-localize with pDCs and proliferate in T cell areas of palatine tonsils. Palatine tonsils reflect classical features of peripheral immune responses in poly-allergic individuals with low expression of T-bet, IFN-? and IL-10 compared to non-allergic controls, without any difference in relatively high FOXP3 expression in both groups. In conclusion, these data show that tonsils are organs of oral tolerance that might be clinically exploited to develop novel ways of immunotherapy for the treatment of allergic diseases and other immune tolerance-related disorders.

In the second part, we focused on chronic airway inflammation in asthma results in structural airway changes including bronchial smooth muscle hypertrophy, globlet cell hyperplasia, subepithelial fibrosis and angiogenesis and the role of rhinovirus in this process. One of the major angiogenic growth factors is vascular endothelial growth factor A (VEGF-A), which is a key regulator of blood vessel growth in airways in asthma patients. IL-32 is a pro-inflammatory cytokine with multiple functions in many tissue cells and is involved in many chronic inflammatory diseases. The aim of the study was to investigate the expression and function of IL-32 in asthma patients. IL-32 is expressed in NHBE cells upon stimulation with IFN-g, TNF-a and Th1 cells and rhinovirus infection. Transfection of NHBE cells with IL-32 siRNA, which resulted in a clear decrease in IL-32 expression, significantly increased the amount of the pro-angiogenic factors VEGF and platelet-derived growth factor (PDGF) secreted by NHBE cells and enhanced tubular formation of HUVECs significantly. IL-32 is detectable in induced sputum from asthma patients and serum IL-32 levels showed a positive correlation with lung function improvement after 20 days intensive asthma therapy. In conclusion, this study demonstrates that IL-32 is involved in asthma pathogenesis and regulates angiogenesis via decrease of VEGF secretion by bronchial epithelial cells.

In WP6: Resolution of inflammation

We have been developing methods for the quantification of lipid mediators in biological fluids, the acquisition of unique standards and the introduction of the nanospray technology in the LC-MS/MS system. Following recommendations from the kick-off meeting, we are expanding method development to additional lipid molecules including prostaglandin D2, prostaglandin E2, eoxin E4, leukotrienne B4 and lipoxin A4. We have also

incorporated the nanospray technology. Early results indicate that increased sensitivity of up to 100-fold can be achieved. In parallel, we started evaluating the effects of certain lipid mediators in animal models of allergic airway disease.

In WP7: Diagnostics

Rhinovirus infections are considered important triggers of asthma but there are no serological diagnostic tests available. WP7 aims to establish diagnostic tests which should allow determining species-specific and cross-reactive antibody responses for a large variety of the most common rhinovirus strains. For this purpose a representative repertoire of recombinant HRV proteins and peptides will be produced and assembled on microarrays. Chips containing micro-arrayed viral proteins and peptides should then allow to measure with small serum samples the complex antibody reactivity profiles in sera which can then be matched with clinical phenotypes and results from virus typing to establish antibody signatures which determine and predict certain clinical courses. This work may form a basis for novel therapeutic or prophylactic intervention strategies for HRV infections.

In WP8: Interventions

Rhinovirus HRV1b, HRV16 and HRV29 was provided by WP2 and target regions were determined in collaboration with the partners in Greece. First step of DNAzyme design was initiated. Therefore cDNA of the viral target RNA (cre-element) was isolated and controlled by PCR technique. The respective cDNA fragments were cloned into the vector TOPO4 and sent out for sequencing to verify sequence integrity. Control of the cloning products is finished. Currently, in vitro cleavage assays are initiated to select active DNAzymes out of fourteen candidates."

PreDicta's website and extranet platform

PreDicta 's public website was launched in January 2011. We would be happy to update it regulary thanks to new data received from all of you: your participation in congresses, important team news, publications etc... So do not hesitate to send your contribution to Dahlia.

The private extranet platform is also fully operational. If you did not get a password, please contact Dahlia.

A specific folder for publications has been created where you shall post a copy of all publication manuscripts at least 45 days prior to the submission date of publication or communication.

Do not forget to acknowledge the Commission funding in all your dissemination acitivities!

More info on:
http://www.extranet-predicta.eu/publications

PreDicta's extranet platform can be reached on
www.extranet-predicta.eu
OR
by Clicking on the button « Private access members » on
www.predicta.eu

PreDicta' series of portraits: *Spotlight on PreDicta researchers*

In this issue: *Susetta Finotto & Katarzyna Niespodziana*

Susetta Finotto

Team: *UKER (Erlangen)*
Status: *Professor*
Area of expertise: *Molecular and cellular immunology of lung diseases*

What is you scientific background?

- Undergraduate studies in Biology with major in molecular biology, University of Padua, Italy.
- PhD at the McMaster University, Hamilton, Ontario, Canada
- Postdoctoral fellowship at the NIAD, NIH, Bethesda.
- Sabbatical at the Brigham Hospital/Harvard School of Public Health, Boston
- Professor and Chief of the Institute of Molecular Pneumology at the University of Erlangen, Germany.

Selected honors and awards

- PHD fellowship exchange program University of Padua-McMaster University
- Fogarty International fellowship NIH

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

- Molecular and cellular research studies on the role of steroids on mast cells.
- Development of new antisense DNA inhibitors of GATA 3 RNA for in vivo treatment of experimental allergic asthma.
- Discovery of a protective role of T-box-expressed in T cells (T-bet) in allergic asthma
- Inhibition of IL-6 signal transduction induced FOXp3+ T regulatory cells which ameliorated experimental asthma.

What is your role in PreDicta?

- Coordination of the clinical and laboratory part (Wp1).
- Studying the influence of Rhinovirus on immunological parameters like IL-17, IL-6, Foxp-3, GATA3 and T-bet in immunocompetent cells from children with and without asthma.

Have you participated in other EU projects?
No, this is my first EU project.

What is your opinion about the PreDicta network?
It is very well coordinated.

Katarzyna Niespodziana

Status: *PhD Student*
Team: *MUW (Vienna)*
Area of expertise: *Allergy and Viral Immunity*

What is your scientific background?

My research interests have spanned a range of topics, from the development of new forms of allergy vaccines based on recombinant fusion proteins consisting of viral carrier proteins and non-allergenic peptides derived from major allergens, to the evaluation of their potential usefulness for the side-effect free therapeutic and eventually prophylactic vaccination against allergy and infectious diseases.

What is your role in PreDicta?

My aims in PreDicta are to establish novel diagnostic tools for the characterization of species-specific and cross-reactive antibody responses to recombinant capsid proteins/ epitopes derived from the most common RV strains. The antibody response profiles can then be used for the determination and prediction of certain clinical courses and thus may form a basis for novel therapeutic or prophylactic intervention strategies.

Have you participated in other EU projects?

PreDicta is my first EU project that I am participated in.

What is your opinion about the PreDicta network?

The PreDicta network provides an excellent opportunity to start new collaborations to tackle questions that can only be addressed in an interdisciplinary approach rather than by individual partners. This approach also facilitates the exploitation of novel research topics and technologies that could not be handled in sufficient detail without collaborating with other scientists.

Kasia had her thesis defence (Development of viral carrier-based allergy vaccines) on March 28th 2011." Congratulations to her!

PreDicta publications: beware of Open Access!

The European Commission has just sent a reminder to all FP7 project coordinators, to remind them of the Open Access rule for all publications issued from FP7 projects.

To summarize briefly, the idea is to make FP7 publications as freely available as possible. Open Acess can thus be defined as « free access over the internet ».

For this, **you should:**

1. deposit an electronic copy of your your manuscripts in an institutional subject-based repository at the moment of publication (mandatory)
2. make them publicly and freely available within 6 months of publication (best efforts required only: if this is not possible, you should provide the Commission with the letter of refusal from the publisher)

The above procedure is often referred to as **Green open access** as it is entirely free.

Publishers may also offer you a « *paid open access* » option, allowing you to deposit your article immediately in open access repositories upon payment of a fee **(gold open access)** .If you chose this option, you will be able to declare publishing costs on your PreDicta cost statement. However, you will not receive any additional budget for this.

The Commission favours Green open access, but Gold open access is also welcome.

Open Access guidelines have been posted on PreDicta's extranet platform.

More info on Open Access on our extranet platform:

http://www.extranet-predicta.eu/publications/Open%20Access.pdf/view

The Open Access rule does not exempt you from posting your abstracts on PreDicta'extranet platform at least 45 Days prior to the submission date of publication or communication.

Next events

Training meeting for the WP1
Date: 20-21 of May 2011
Place: Allergy & Immunology Research Center, University of Athens, Athens, Greece
Organized by Vicky Xepapadaki and Maria Passioti
The training meeting for WP1 has been planned for May 20-12, as it is of vital importance to have a common approach between all the participating teams. Two persons from each center, one for the clinical and one for the laboratory part will participate following the respective training sessions, one for the recruiting clinicians and one for the scientists performing the PBMCs' cultures.
This training meeting is an excellent opportunity to discuss any questions / clarifications regarding the protocol!

PreDicta Meeting during EAACI Congress, Istanbul 2011
Date: 13 of June 2011, 17:00-19:00
Place: Lutfi Kirdar Convention and Exhibition Centre (ICEC) ,Istanbul, Turkey
The annual EAACI Congress in Istanbul is now approaching. Many of the scientists with interest in the field of Allergy and Immunology will be there, making this event an ideal opportunity for a summer PreDicta meeting!
PreDicta first clinical and laboratory results as well as the first related publications can be reviewed and discussed.
We are looking forward to seeing you there!