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News and EFIS

Immunology in the heart of the High Tatra mountains

The concept of the Tatra Immunology Conference series was put together in the early 1990s by Juraj Ivanyi (London, UK) and Georg Wick (Innsbruck, Austria), with the support of the Czech, Slovak, and British Immunology Societies and the Austrian Society for Allergology and Immunology, to promote the scientific re-emergence of the Central/Eastern European region. Since its first meeting in 1994, the aim of this Conference has been to allow young scientists and trainees from this region to meet with world class scientists and have the opportunity, not only to listen to their cutting-edge lectures but also to continue with rather informal discussions during the mid day hiking trips to the surrounding spectacular mountains, rustic villages, or castle ruins (Fig. 1).

Since 1998, the Tatra Conference has been held as a regular EFIS meeting, receiving monetary support since 2008 from the *European Journal of Immunology* by way of the EFIS-EJI partnership, leading it to be called the EFIS-EJI Tatra Immunology conference. It is currently held every two years, with a schedule that includes morning and late-afternoon lectures by invited speakers, poster presentations by other participants (Ph.D. students, postdocs, and medical residents), and informal discussions; all still combined with the extended midday recreational activities, i.e. hiking trips (Fig. 2). The aim of the organizers is to have a style similar to that of the Gordon Conferences. The number of participants is limited to approximately 120 (Fig. 3), with the majority of the students and trainees coming from the Czech Republic, Slovakia, and Austria, supported by travel grants provided by EFIS-EJI, national immunology societies, and by the participants' institutions; however, there is increasing interest among students from other countries such as Germany, The Netherlands, and UK to participate. Sadly, despite our best efforts, intense advertising, and generous travel grants offered by EFIS-EJI, we fail to attract large number of participants from Eastern Europe and post-Soviet countries.

Figure 1. Mid day hiking trip during the Tatra Conference.

The 3-day scientific programmes at all EFIS-EJI Tatra Conferences have had sessions ranging from fundamental to clinical immunology; however, in the past few meetings, the major goal of the scientific program has been to document the importance of basic and clinical research for the development of novel diagnostic and therapeutic strategies in clinical medicine. This report highlights some of the key presentations of the 9th EFIS-EJI Tatra Immunology Conference held at Štrbské Pleso in the High Tatra Mountains, Slovakia; from September 4–8, 2010, and organized by myself together with Václav Hořejší (Czech Immunological Society), Falk Nimmerjahn (Erlangen, Germany), Stanislava Blažíčková, Zuzana Popracová, Zuzana Polčíková (Slovak Immunological Society), and Hannes Stockinger (Austrian Society for Allergology and Immunology).

Recent advances in basic immunology

To begin the conference, Kevin Woollard (London, UK) described current models of the development and functions of mononuclear phagocytes. Current models propose that blood monocytes, many macrophage subsets, and most DCs originate in vivo from hematopoietic stem cell (HSC)-derived progenitors with myeloid-restricted differentiation potential. Successive commitment steps in the bone marrow include common myeloid progenitors (CMPs), granulocyte-macrophage precursors (GMPs), and macrophage/DC progenitors (MDPs). Although many macrophages and DC subsets are renewed from bone marrow progenitors, there are notable exceptions. For example, neither microglia nor Langerhans cells (LCs) are dependent on the bone marrow for their renewal in the steady state and possibly during inflammation.

Blood monocytes have been considered as precursors for macrophages and dendritic

Figure 2. Relaxing at the summit of the Solisko hill.

Figure 3. Participants of the 2010 Tatra Conference.

cells but, as Kevin Woollard explained, evidence now indicates that blood monocytes are instead effectors of the inflammatory response. Human $CD14^+$ monocytes, which can express CD16 when activated, specialize in phagocytosis and production of reactive oxygen species (ROS), and secrete inflammatory cytokines in response to a broad range of microbial cues. In contrast, human monocytes that lack CD14 but express CD16 ($CD14^{dim}$ monocytes) are weak phagocytes and do not produce ROS or cytokines in response to cell surface TLRs. Instead, they selectively produce the pro-inflammatory cytokines TNF, IL-1b, and CCL3 in response to viruses and immune complexes containing nucleic acids via a unique TLR7-8/MyD88/MEK pathway [1]. $CD14^{dim}$ monocytes may be involved in the innate local surveillance of tissues and the pathogenesis of autoimmune diseases.

Diana Dudziak (Erlangen, Germany) then presented data on dendritic cells (DCs) as master regulators of the immune response. DCs in either an immature or mature state are capable of presentation of antigen; T cells recognizing peptide MHC-complexes on immature DCs undergo deletion or anergic responses, whereas T cells recognizing peptide MHC complexes on mature DCs undergo proliferative responses, leading to T cell memory, indicating that immune responses are tightly regulated by the state of DCs. Given that DCs are very potent antigen presenters, the idea arose that it might be possible to target antigens to DCs in vivo as a new vaccination strategy. By targeting antigens to the main murine DC subpopulations it was shown that antigen-loaded $CD11c^+CD8^-$ DCs induce a pronounced CD4 helper T-cell response whereas antigen loaded $CD11c^+$ $CD8^+$ induce a prominent CD8 T-cell response in C57BL/6 mice [2]. By antigen targeting of DC subpopulations under tolerogenic conditions de novo differentiation of peripheral antigen-specific regulatory T cells was induced when the antigen was presented by $CD11c^+CD8^+$ DCs; however, after the transfer of antigen-specific regulatory T cells into mice that had been targeted with antigen to $CD11c^+$ $CD8^-$ DCs, the transferred regulatory T-cell population was found to be expanded in vivo. These results further indicate that the specific antigen presentation by different DC subpopulations might influence the outcome of immune reactions.

Jens Geginat (Milan, Italy) nicely described the identification and characterization of two distinct subsets of human $Foxp3^-$ IL-10-secreting T cells with regulatory properties [3]. IL-10 produced by $CD4^+$ T cells is important to prevent autoimmunity and immunopathology, but the in vivo identity of adaptive IL-10-producing regulatory T cells in humans was until recently unknown. Jens Geginat showed that the $CCR6^+IL\text{-}7R^{hi}$ T-cell population contains not only Th17 cells but also memory cells that secrete suppressive IL-10 upon suboptimal TCR stimulation and with autologous DC; however, the same cells also produce CD40L, IFN-γ, and IL-2 following optimal TCR stimulation and with a relevant recall antigen, which is similar to the response of conventional memory T cells, suggesting that the cells have a context-dependent regulatory

function. A subset of IL-10-producing Th1 effector cells, which suppress T-cell proliferation by an IL-10-dependent mechanism, was also identified in the $CD4^+CD25^-IL\text{-}7R^{lo}$ T-cell population. These effector cells express high levels of CTLA-4, and are anergic in vitro but proliferate in vivo presumably in response to persistent antigens. As the identified memory and effector-like T-cell subsets show different requirements, kinetics, and stabilities of IL-10 production, Jens Geginat proposed that they have different functions and might inhibit different types of immune responses.

Naturally occurring regulatory T cells (Tregs) have been shown to control immune responses to self and non-self. Muriel Moser (Brussels, Belgium) discussed the regulation of Th1 cells by naturally occurring and adaptive Tregs. It has previously been shown that depletion of natural Treg before immunization with antigen-pulsed dendritic cells (DC) results in increased Th1-type responses characterized by high levels of IFN-γ production and CTL activity. The mechanism by which Tregs control the development of Th1-like responses, including the role of two Th1-prone factors, IL-12 and CD70, has also been examined. In vivo Treg depletion was found to lead to increased IFN-γ production in both wild-type and IL-12 p40-deficient mouse strains, suggesting that the ability of Tregs to down-modulate Th1 responses is largely IL-12- and IL-23-independent. In marked contrast, neutralizing antibodies to CD70, a membrane-associated TNF family member, prevented the ability of Treg depletion to increase IFN-γ production. In vitro experiments demonstrated that Tregs inhibit CD70 expression in a contact-dependent manner and, although the suppressive mechanism is still unclear, it may involve a phenomenon of (trans)-endocytosis because $CD27^{-/-}$ Tregs failed to downregulate CD70 in vitro. These observations indicate that natural Tregs control Th1 cell development by predominantly interfering with the CD70/CD27 pathway.

Tomáš Brdička (Prague, Czech Republic) presented new data on the regulation of Src-family kinases (SFKs) in leukocytes. SFKs are regulated by phosphorylation of their inhibitory and activatory tyrosines, with the outcome depending on the complex interplay between the activities of several phosphatases, kinases, and adaptor proteins. In leukocytes the prominent role is played by receptor-like protein tyrosine phosphatase CD45 which is able to both positively and negatively regulate SFKs by dephosphorylating their negative and positive regulatory sites respectively. Activatory function appears to have a dominant role as judged from the studies of CD45-deficient mice and humans.

CD148 is another receptor-like protein tyrosine phosphatase (PTP), which acts as a suppressor in solid tumors by inhibiting transduction of mitogenic signals. In hematopoietic cells, CD148 inhibits T-cell receptor signaling by dephosphorylating several key signaling molecules, including LAT and PLCγ. On the other hand, Tomáš Brdička's data suggest that in B cells and macrophages CD148 augments immunoreceptor signaling via dephosphorylation of the C-terminal tyrosine of SFKs. Thus, it seems that CD148 may have the opposite function in T cells as compared with other leukocytes. To reconcile this controversy, Tomáš Brdička's group analyzed the function of CD148 in human T-cell lines in a CD45-deficient setting. It was found that under these circumstances CD148 is able to dephosphorylate inhibitory tyrosines of SFKs and thus activate these kinases and rescue signaling defects caused by CD45 deficiency. The study suggests that dual inhibitory/stimulatory function may be a common principle governing the signaling by different receptor-like PTPs.

Gerhard Schütz (Linz, Austria) introduced the methodology behind the fascinating world of single molecule microscopy. Current scientific research throughout the natural sciences aims at the exploration of structures with dimensions between 1 and 100 nm. In the life sciences, the diversity of this nanocosm attracts more and more researchers to the emerging field of nanobiotechnology. Gerhard Schütz explained how to obtain insights into the organization of the cellular compartments by single molecule experiments. He presented results on the interaction between antigen-loaded MHC and the T-cell receptor, looking directly at the interface region of a T cell with a mimic of an antigen-presenting cell. He also presented studies of the interaction between CD4 – the major coreceptor for T cell activation – and Lck, a tyrosine kinase important in early T cell signalling.

Tumor immunology and cancer immunotherapy

It was an honor to have the current EFIS President Catherine Sautès-Fridman (Paris, France) to start the session on tumor immunology. She illustrated the double role of the immune response in the outcome of cancer, presenting experimental data obtained from lung cancer patients [4]. The density of mature DC, a cell population which homes exclusively to the T-cell areas of BALT, forming synapses with naive T cells, correlates with prolonged survival in patients with early-stage NSCLC. Catherine Sautès-Fridman hypothesized that tumor antigens that are continuously sampled and processed by DC activate T cells in situ, thereby increasing the efficiency of the immune response. On the other hand, TLRs allow for recognition of pathogens and trigger inflammatory responses through activation of NF-κB. The human lung is in contact with inhaled airborne pathogens and, via expression of a large panel of TLRs, the airway epithelial cells represent the first barrier against invading microbes. Several studies strongly suggest that chronic inflammation increases the risk of carcinogenesis. As lungs are frequently exposed to RNA viruses that are recognized by TLR7 and TLR8, the expression of TLR7 and TLR8 by tumor cells in human lung cancer in situ and in cell lines was investigated. Stimulation with TLR7 or TLR8 agonists leads to atypical NF-κB activation, up-regulation of Bcl-2 expression, increases tumor cell survival, and induces chemoresistance. Altogether, these data emphasize that TLR signalling occurring during infection in lung cancer patients could directly favor tumor development.

Peter Brossart (Bonn, Germany) then discussed current strategies of cancer immunotherapy, focusing on his groups' studies using DCs presenting tumor antigens [5]. DCs are the most powerful antigen presenting cells with the unique ability to initiate and maintain primary immune responses. Due to a better understanding of DC differentiation and function, and the establishment of protocols for the generation of DC in vitro under GMP conditions, vaccination strategies were developed to treat patients with malignant diseases. Peter Brossart presented data from a recently finished clinical trial using autologous mature DCs pulsed with MUC1-derived HLA-A2 binding peptides. This approach resulted in the induction of clinical and immunological responses in vaccinated patients with metastatic renal cell carcinoma. Currently, the Brossart group is characterizing novel tumor antigens and analyzing several approaches to improve

the efficiency of such vaccines by utilizing in vitro transcribed RNA that code for defined tumor antigens or combinations with tyrosine kinase inhibitors.

Peter Šebo (Prague, Czech Republic) delivered a rich and fascinating overview of *Bordetella* adenylate cyclase toxin (ACT) and suggested its possible use in cellular therapies. ACT targets myeloid phagocytes bearing the $\alpha_M\beta_2$ integrin CD11b/CD18 (Mac-1 or CR3), such as neutrophils, macrophages, or dendritic cells (DC, CD11b^{high}) [6]. ACT penetrates across the cell membrane, promotes an influx of calcium ions, binds cytosolic calmodulin, and converts ATP to cAMP, thus causing phagocyte impotence. In DCs, partial maturation by ACT is induced that compromises their capacity to stimulate T cells. The AC domain of detoxified ACT, having the enzyme activity ablated genetically (dACT), in turn, exhibits an amazing capacity to accommodate foreign T-cell antigens and convey them into the cytosol of dendritic cells both in vitro and in vivo. This allowed the development of dACT toxoids into a particularly efficient tool for antigen delivery for cytosolic processing and MHC class I-restricted presentation to cytotoxic CD8$^+$ T lymphocytes. Moreover, a fraction of dACT molecules is taken up by clathrin-dependent uptake, which enables endosomal antigen delivery for the presentation on MHC II molecules; dACT thus allows efficient induction of prophylactic, as well as therapeutic, antigen-specific CD4$^+$ and CD8$^+$ T-cell immune responses. This allows the use of ACT technology for antigen delivery and tumor immunotherapy.

Diagnosis and treatment of autoimmune diseases and allergies

Autoimmune diseases are common and debilitating, but their severe manifestations could be reduced if biomarkers were available to allow individual tailoring of the potentially toxic immunosuppressive therapy required for their control. Clinically useful biomarkers have been identified using DNA microarrays in cancer but not autoimmunity. Ken Smith (Cambridge, UK) showed that transcriptional profiling of purified CD8 T cells, but not unseparated T cells, identifies two distinct patient subgroups predicting long-term prognosis in four different autoimmune diseases: anti-neutrophil cytoplasmic antibody (ANCA)-associated vasculitis, systemic lupus erythematosus, ulcerative colitis, and Crohn's disease. Ongoing work is also examining renal transplantation, and the underlying mechanism driving these transcriptional signatures. Ken Smith showed that genes defining the poor prognostic group are enriched for those of the IL-7R pathway, TCR signaling, and, in some diseases, those expressed by memory T cells [7]. These subgroups can be identified by measuring expression of only three genes, raising the prospect of individualized therapy and suggesting novel potential therapeutic targets for autoimmunity.

Mattias Collin (Lund, Sweden) suggested antibody glycan hydrolysis as a novel therapy against autoimmunity. The enzyme EndoS from *Streptococcus pyogenes* is an immunomodulatory molecule hydrolyzing the conserved glycans in the effector part of immunoglobulin G (IgG) [8]. EndoS is remarkably specific for IgG, and hydrolysis has profound effects on IgG effector functions. EndoS pretreatment of IgG, or direct administration to animals with experimental antibody-mediated autoimmune diseases, inhibits development of disease or cures animals from established disease. The properties of EndoS make it a unique experimental tool and an attractive alternative to current therapies of conditions involving pathogenic antibodies, including antibody-mediated autoimmune diseases and acute transplant rejections. Mattias Collin described ongoing studies of the biotechnological potential of EndoS, as well as the outcomes of EndoS treatment in several, both passive and active, animal models of autoimmunity.

Jörg Köhl (Lübeck, Germany) presented data on novel roles of complement in the regulation of adaptive immunity. Data obtained in the past few years provide evidence that translation of complement-derived sensing signals from the fluid phase into distinct cellular responses is an important mechanism by which the innate immune system regulates adaptive immune responses. The small cleavage fragments of C3 and C5, the anaphylatoxins (AT) C3a and C5a, and the activation of their corresponding AT receptors (ATR), the C3a receptor (C3aR), the C5a receptor (C5aR) and C5L2, on antigen presenting cells (APC) are of particular importance in this respect. Activation of ATRs on dendritic cells (DC) and macrophages regulates the activation profile of APCs either autonomously or by modulation of TLR-mediated activation of DCs and macrophages. This regulatory impact is critical for the differentiation of CD4$^+$ Th cells toward Th1, Th2, Th17, or Treg cells in models of allergy, autoimmunity, and infection. Jörg Köhl presented data showing novel roles for the ATR in the development of pathologic immune responses in allergic asthma and two models of autoimmune diseases, anti-GBM nephritis and autoimmune arthritis.

Fatima Ferreira (Salzburg, Austria) described modern strategies for developing safe and effective allergy vaccines. Allergen-specific immunotherapy (SIT) is an effective treatment for allergic rhinitis and asthma; however, the problems associated with SIT (e.g. use of extracts that are difficult to standardize, induction of new IgE specificities, IgE-mediated side effects, etc.) hamper its wider use. The use of recombinant allergens that are structurally and immunologically equivalent to their natural counterparts offers important advantages over the use of natural extracts, especially because recombinant allergen preparations contain defined amounts of the active component and can be standardized. Efforts are being undertaken to develop hypoallergenic molecules in order to diminish the risk of IgE-mediated side effects. Several strategies have been used to generate structurally altered allergens with reduced or abolished IgE antibody binding capacity. Such structural modifications might have different effects on allergen structure and consequently not only on the allergenicity but also on the immunogenicity of the molecules. Fatima Ferreira's group has performed extensive studies investigating how structural manipulations of allergens impact on immune responses. Their results indicate that folding, aggregation status, and stability to degradation by DC-derived endolysosomal proteases have profound effects on the immune responses elicited by candidate allergy vaccines.

Concluding remarks

In addition to the talks by the invited speakers, which I have discussed above, one afternoon session consisted of oral presentations of six selected posters. This session represented a true highlight of 2010's conference, not only because of the great and enthusiastic presentation by the selected trainees but, in large part, due to the fantastic chairing of this session by Adrian Hayday (London, UK) who elicited truly electrifying and lively discussions. This session was very well received and,

based on the comments from the participants, we intend to extend this session in future EFIS-EJI conferences.

The EFIS-EJI Tatra Immunology Conference has become a tradition and it provides an important get-to-know opportunity for the young immunology trainees from not only the Central/Eastern Europe region but beyond. We hope for continuous EFIS-EJI support for future meetings, which is indispensable as it provides travel grants for a significant number of young immunologists who attend the conference. The next conference is planned for September 2012 and the details will be posted on http://www.img.cas.cz/tatra/ approximately one year in advance of the meeting. Perhaps we will see you there.

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Day of Immunology 2011 – Make it YOUR day!

In preparation for this year's Day of Immunology (29 April 2011), the Day of Immunology 2011 website (http://dayofimmunogy.org) is now live. Importantly, a guestbook section (http://www.dayofimmunology.org/guestbook_view) has been added (see the section, Participate!). You are invited to share your ideas, thoughts and experiences regarding public relations in immunology with the scientific community via this new section. You are also most welcome to post a short feedback, comment or a summary of your Day of Immunology activities. Let the immunological community know what you have planned for the Day of Immunology 2011 at:

http://dayofimmunogy.org