Recent findings on innate and adaptive immunity

Circe Mesa[1], Yraldo Bello[2], ✉ Celia Fernández-Ortega[3]

[1] Vaccine Department, Center of Molecular Immunology,
216 esq. 15, Atabey, Playa, CP 16040, Ciudad de La Habana, Cuba
Fax: (53-7) 272 0644
[2] Department of Clinical Trials
[3] Department of Cell Biology, Center for Genetic Engineering and Biotechnology, CIGB
Ave. 31 e/ 158 y 190, Cubanacán, Playa, AP 6162, CP 10600, La Habana, Cuba
Fax: (53-7) 271 4764; E-mail: celia.fernandez@cigb.edu.cu

The 12th International Congress of Immunology and the 4th Annual Conference of FOCIS held in Montreal, Canada on July 2004 (ICI-2004) offered contributions on topics such as stem cell and bone marrow transplantation, vaccines, infectious and autoimmune diseases, tumor biology and others. Important subjects of basic immunology were also discussed in order to link the knowledge on the immune system with the achievement of better treatments for many diseases.

The studies of dendritic cell (DC) biology, their function and key role on the immune system regulation were some of the basic research areas discussed in the meeting. These subjects were not only programmed in specific oral and poster sessions but were also brought up in most of the talks, refering to the role of dendritic cells as the initiators of the immune responses elicited in many diseases including HIV infection.

The meeting was huge; every participant was physically able to attend up to 15% of the major-symposiums and only 4% of the micro-symposiums. Therefore, this report will cover only a few conferences regarding innate immunity, antigen processing and presentation by DC, their role on regulating immune responses, and immunotherapy and immunopathology of HIV.

The innate immune system is an ancient mechanism of host defense found in essentially every multicellular organism, from plants to humans. In invertebrates, it is the only mechanism of defense. Vertebrates also developed an adaptive immune response, however, the innate immune system is essential for instructing the cells of the adaptive system (T and B cells) by presenting the antigen in the context of appropriate costimulatory molecules. The innate immune system was developed not only to discriminate self from non-self but more importantly, to discriminate *infectious* non-self from *innocuous* non-self.

DC and other cells of the innate immune system sense and respond to microbial products via the Toll-like receptor (TLR) family. TLRs are an evolutionarily conserved family of cell surface molecules that participate in innate immune recognition of pathogen-associated molecular patterns (PAMPs). PAMPs are generally unique, chemically diverse products with conserved motifs that are produced by microorganisms.

In the Innate Immunity session of the ICI-2004, scientists that have made major contributions to this topic shared their latest advances. Dr. Jules A. Hoffmann (France) the first to describe, in 1996, the Toll as pattern recognition receptor in *Drosophila*, opened the session with an overview of *Drosophila* Immunity. The followeing conferences were related to these receptors and how they respond to infections. Dr. Bruce Beutler (USA), described the five year work to develop 53 000 mutant mice by irradiation. They found 12 mutant mice va-riants in which macrophages had defective responses to LPS, dsRNA and CpG. He and his colleagues found a gene called Lps2 responsible for this deficiency. The expression of this gene blocked TLR3 and TLR4 and abolished MyD88 independent events.

Related to TLRs, Dr. Shizuo Akira (Japan) confirmed the idea that each TLR displays a difference in the expression pattern, intracellular localization, and signaling pathway, resulting in distinct immune responses. He stressed the signaling activated after microbial recognition as the mechanism used to define gene targeting. An example of this is the induction of IFNα production after TLRs engagement. A subset of TLRs, TLR7, TLR8 and TLR9, induces antiviral res-ponses by producing IFNα. Production of IFNα is dependent on the Toll-interleukin-1 receptor domain-containing adaptor MyD88. During his conference Dr. Akira showed that MyD88 forms a complex with the transcription factor IRF7 but not with IRF3. The death domain of MyD88 interacts with an inhibitory domain of IRF7, and this interaction results in the activation of the IFNα-dependent promoters.

Markus Frey and collaborators from Germany indicated that IFNα induction and subsequent signaling is not confined to TLR-3 and TLR-4 signaling but is a general property of TLRs. There are however quantitative (amount of IFNα) and qualitative (MyD88 dependency) differences between the TLRs. Common as well as alternative TLR signaling pathways are strongly interconnected and influence each other.

One of the most important conferences in the Innate Immunity session was related to the interaction of TLRs with CD4 T cells, specially regulatory CD4 T cells (Treg). This issue was exposed by Dr. Ruslan Metzitov (USA), who started these studies two years ago. His data confirmed that DC mediated CD4 T cell activation depends on TLRs. This phenomenon is not only due to the induction of costimulatory molecules on DC but also to TLR-mediated blocking of Treg suppression. Dr. Metzitov explained that this mechanism is independent of costimulation and instead it is mediated by IL-6 produced by DC in response to TLR activation. IL6 in turn, affects antigen specific T cells and makes them refractory to suppression by Treg cells. In this respect he addressed the question: would Treg depletion restore T cells response in the

✉ Autor de correspondencia

absence of TLR activation. Experiments on immunization with human serum albumin (HSA) (this antigen does not induce DC maturation) demonstrated in Treg depleted mice that there was no T cell activation in the absence of TLR-ligands or the absence of Treg cells. This experiment was also performed in $CD28^{-/-}$ mice. He concluded that the requirement for a costimulatory signal can not be bypassed by the depletion of Treg cells.

The closing conference of this session was by Dr. Arthur Kreig (USA) who has made major contributions in CpG. Unmethylated CpG dinucleotides mimic the immune stimulatory activity of bacterial DNA in vertebrates and are recognized by TLR9. Here Dr. Kreig made an overview of CpG as an adjuvant for allergy, infectious and cancer vaccines, but he employed on cancer immunotherapy. He discussed the strategy of combining vaccination with chemotherapy in which the abrasive therapy with drugs could a crea-te space in the T cell repertoire or diminish Treg. He showed preclinical experiments in which the treatment with Cyclophosphamide (Cy) and CpG increase the survival of mice with 19 day tumors. These results encouraged them to start a randomized clinical trial in patients with NSCLC (Non Small Cell Leing Cancer).

The activation of DC through TLRs is the molecular mechanism by which DC contribute to the induction of peripheral T cell tolerance. The session related to the Immunobiology of DC brought together the most important scientists in this field. Dr. Ralph Steinman (USA), chairman of the session, introduced the topic emphasizing the many different subsets of DC, their features, but more importantly their different roles.

The second conference, by Dr. Michel Nussenzweig (USA) attracted the audience`s attention. To show the differences between steady and activated DC they cloned EYFP (green fluorescent protein) under the CD11c promoter and made a transgenic mouse. With this technology they were able to follow up, in a complete mouse, the activated (brighter) and steady state (less brighter) DCs. They assessed, migration, motility, life time, and DC-T cell interaction.

Dr. Paola Ricciardi-Castagnoli the Italian scientist that discovered IL2 production by DC explained its relevance for innate responses. Using transcriptome analysis they have compared the IL2 production by DC treated with different pathogens. They found that DC treated with *Listeria monocyto-genes* (Lm), *Schistosoma mansoni* (Sm) and *Leishmania mexicans* (LM) produced IL2 at 4, 12, and 4 hours respectively and analyzed the relevance of the kinetics for DC interaction with NK and NKT cells. She also mentioned that some stimuli such as zymosan, yeast, LPS and CpG induce much more production of IL2 that activated T cells. *In vitro* and *in vivo* experiments demonstrated that NK and NKT cells failed to produce IFNα when were activated with DC derived from $IL2^{-/-}$ or $RAG^{-/-}$ mice.

One of the most interesting lectures in that session was given by Dr. Yvette Van Kooyk (Netherlands). She described the role of C-type lectins on self and non-self antigen recognition on DC. Residues of the Lewis carbohydrate antigen or the presence of the CD16 molecule on polymorph nuclear cells (PMN) such as granulocytes makes them susceptible to recognition by C-type lectins receptors on DC. Dr. Van Kooyk demonstrated that immature DC and granulocytes form clusters through DC-SIGN (C-type lectins receptor) and this interaction induces DC maturation and IL12 production if the PMN were previously activated with LPS or TNFα. She also showed the opposite results when incubating DC with mycobacterial mannosylated lipoarabinomannan (ManLAN), a soluble factor secreted by macrophages after infection. ManLAN binds also to DC-SIGN on DC but inhibits DC maturation and induces IL10 production. These results suggested that DC-SIGN, upon binding with ManLAM, interferes with TLR-mediated signals. Blocking antibodies against DC-SIGN reverse the ManLAM-mediated immunosuppressive effects.

The relevance of these receptors on Th1-Th2 polarization was addressed with the human gastric pathogen *Helicobacter pylori*, which spontaneously switches lipopolysaccharide (LPS) Lewis (Lew) antigens on and off. She reported that Le(+) *H. pylori* variants are able to bind to the C-type lectin DC-SIGN present in gastric DCs, and demonstrated that this interaction blocks T helper cell (Th)1 development. In contrast, Le(-) variants escape binding to DCs and induce a strong Th1 cell response. Both conditions induced Th1 polarization when treated with the anti-DC-SIGN monoclonal antibody.

The controversial origin of plasmacytoid dendritic cell precursors (pDC) was addressed in the session by Dr. Yong-Jun Liu (USA). However, of greater interest was a novel epithelial cell-derived cytokine and its potential role in the induction of allergic inflammation. He presented recent evidence that human thymic stromal lymphopoietin (TSLP), a novel IL-7-like cytokine, might represent an early trigger of the allergic immune cascade. TSLP-activated human DCs produce Th2-attracting chemokines such as TNF but not IL-12 or IL10 and induce naive $CD4^+$ and $CD8^+$ T cell differentiation into effectors cells with a typical pro-allergic phenotype. TSLP is produced by human epithelial, stromal, and mast cells. It is highly expressed by the keratinocytes of atopic dermatitis but not in other types of skin inflammation. He stated that epithelial and stromal cell derived TSLP might represent one of the factors initiating the allergic responses, and could be a target for a curative therapeutic approach to allergy.

The biology of DC on HIV infection was also brought up. During the session on the Immunotherapy of HIV Dr. Gessani from Italy suggests the existence of multiple relationships between DCs and T cells. DCs play a crucial role in HIV-1 infection, particularly in the early stages of the disease, when they are actively involved in viral transmission. Although viral strains found in acutely infected individuals are predominantly R5, the emergence of X4 variants later in the course of the disease precedes the rapid decline in $CD4^+$ T cell counts and progression to AIDS. The results from Gessani and colleagues indicate that factors released in the microenvironment from infected T cells can modulate the susceptibility of DCs to HIV infection, thus contributing to viral spreading.

To complete the ideas on how DC control immunity and tolerance Dr. Jacques Banchereau (USA)

compared serum from patients with autoimmune diseases such as systemic lupus erythematosus (SLE) and rheumatoid arthritis (RA). Their results demonstrate profound alterations of DC homeostasis in SLE. High levels of IFNα in the serum of SLE patients coexist with decreased numbers of cells producing IFNα, i.e., plasmacytoid dendritic cells (PDCs). Decreased numbers of circulating DCs correlate with increased levels of soluble TNF receptors. The opposite was found when RA patients were analyzed. They proposed a model where autoimmunity can be viewed as a dynamic system driven by opposite vectors: IFNα/β and TNF. These cytokines drive the differentiation of distinct types of DCs, TNF-DCs, or IFN-DCs, which present different antigens lea-ding to distinct autoimmune responses. When ba-lanced, both cytokines synergize in protective immunity. When one of the cytokines prevails, autoimmunity occurs, Type I interferons (IFNα/β) playing a major role in SLE, and TNF playing a major role in RA. This model complements the Type 1/ Type 2 paradigm. Therefore, immunity can be viewed as a dynamic system driven by two sets of opposite vectors: IFNα/β / TNF and IFNγ / IL-4.

Another highly debated topic was the role of lipid raft in several immunological events. Lipid rafts are membrane microdomains, composed of cholesteroland sphingolipid-rich subdomains in the plasma membrane, whose existence has been involved in the assembly and aggregation of receptor complexes, which serve to orchestrate downstream signaling events. Protein reorganization at the immunological synapse between a T cell and an antigen presenting cell (APC) plays a critical role in initiating a cellular immune response. Arrays of clustered major histocompatibility complex (MHC)-peptide complexes and costimulatory molecules on APCs are key features of a mature synapse. There is evidence that not only agonistic MHC II-peptide complexes, but also endogenous self-peptides presented on APCs may contribute to synapse formation and T cell activation. The clue to these findings may be that APCs pre-organize MHC-peptide complexes in lipid microdomains prior to their engagement in the synapse. Using density gradient fractionation and imaging technologies, Harold Kropshofer and colleagues from Germany showed that APCs harbor two types of membrane microdomains, denoted as lipid rafts and tetraspan microdomains (TS domains). TS domains are characterized by the combined presence of the tetraspanins CD9, CD63, CD81, CD82, the costimulatory molecule CD86 and HLA class II-peptide complexes, and appear to be formed in endosomal / lysosomal loading compartments and conclude that TS domains of APCs are critical means to optimize and tune antigen-specific T helper cell activation. Ronald B. Corley and colleagues suggest that lipid raft microdomains may serve not only to orchestrate receptor signaling, but to sequester signaling components from one another to prevent receptor-mediated signaling from occurring. The potential role that receptor-ligand sequestration plays in the B cell lineage was discussed.

The studies on HIV/AIDS during the meeting cover other interesting topics like strategies for new anti HIV therapies. In this regards Elena Perez from USA hypothesized that the expression of a membrane bound gp41-derived fusion inhibitor would confer HIV-1 resistance to primary $CD4^+$ T cells. She and her colleagues designed a strategy for the expression of gp41-derived fusion inhibitors on the surface of primary $CD4^+$ T cells, using a self-inactivating lentiviral vector for gene delivery. They showed that the methodology confers significant HIV resistance to a virulent CXCR-4 tropic strain of HIV-1. They hope to develop this into a clinical trial of adoptive immunotherapy for HIV infection. Barbara Papadopoulou and collaborators from Canada developed a new live parasitic vector (Leishmania) non-pathogenic to humans as a recombinant HIV vaccine. This vector targets APCs and secondary lymphoid organs. It also induces antigen presentation by major histocompatibility complex (MHC) I and II pathways as well as $CD4^+$ and $CD8^+$ T cell activation. The vector was genetically engineered to express biologically active HIV-1 antigens capable of inducing specific antiviral cell-mediated immunity, to target with a higher efficiency and specificity APCs, mainly DCs and to serve as an immunostimulatory adjuvant capable of activating innate and adaptive immune responses. The results show high levels of biologically active HIV-1 Gag and $CD4^+$ T cell prolife-ration in response to HIV-1 antigens in both BALB/c and C57BL/6 mice after vaccination indicating the development of specific T cell-mediated responses against HIV Gag. This vaccination strategy should maximize the potential for targeting HIV immunogens to APCs, and enhance their MHC/antigen presentation functions and persistence which may increase the magnitude and quality of $CD4^+$ Th cell and $CD8^+$ CTL responses.

T regulatory cells were continuously debated in the congress. There is accumulating evidence for the existence of $CD4^+CD25^+$ T regulatory cells (T reg) in the peripheral blood of humans. Recent data suggest that they can be generated in the periphery and play a role in the control of immunopathologies associated with chronic inflammation and persistent infections. Foxall and collaborators from Portugal present a characterization of circulating $CD4^+CD25^+$ T cells in HIV1 and HIV2 infected individuals in comparison with healthy controls. Progressively increasing levels of activation were characteristic of both HIV1 and HIV2 infections. However, HIV-infected and healthy individuals did not show significant intercohort differences in the frequency or activation profile of T reg, implying that these cells may be independently regulated, and concluding that further functional studies are required to fully assess the importance of these cells in the context of HIV immunopathogenesis.

Undoubtedly, one of the most highly attended lectures in the meeting was delivered by Dr. Fauci from USA. He discussed about the steady stream of emerging and reemerging infectious diseases in recent years and the case of the AIDS pandemic as an emergent disease. He focuses on recent findings from his lab on how the aberrant immune activation drives HIV pathogenesis. That activation has a deep impact on the resting $CD4^+$ T cells viral reservoir. These cells isolated from viremic patients are weakly different from aviremic patients measuring spontaneous virus release

in vitro. Early results from this group indicated that the hyperactivation induced by HIV lead to the dysfunction of B cells. Although B cells are not infected with HIV, the virus apparently induces indirect effects on a subset of cells resulting in aberrant activation and finally cell death. This contributes to the widespread immune cell depletion and dysfunction characteristic of HIV infection. Studying the effects of the HIV envelope protein on normal lymphocytes they found that the envelope proteins induce the expression of cellular genes, including genes associated with the enhancement of HIV replication as NFAT that codes for a transcription factor that regulates the transcription of cytokine genes needed for immune cell activation as well as genes needed for HIV replication . This induction of gene expression occurs in the absence of the expression of the classical markers of cellular activation. That induction requires coordinate signaling through the CD4 receptor and the appropriate co-receptor. The envelope proteins that bind to the CCR5 co-receptor induce a different set of genes than those that bind to the CXCR4 co-receptor.

HIV pathogenesis paradoxically involves both direct and indirect effects of HIV that constitute a relentless cycle of aberrant immune activation. HIV can directly infect and lead to the death of $CD4^+$ T cells, but it can also indirectly activate $CD4^+$ T cells. The complex process of aberrant immune activation associated with HIV infection and replication is the main driving force of the pathogenesis of HIV disease.