Tolerance, Autoimmunity and Immune Regulation: Report from the Keystone Symposia, Breckenridge, Colorado (USA)

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«TOLERANCIA, AUTOINMUNIDAD Y REGULACIÓN INMUNOLÓGICA»:
RESUMEN DEL SIMPOSIO KEYSTONE, BRECKENRIDGE, COLORADO (USA)

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RESUMEN
Los grupos internacionales más relevantes en tolerancia inmunitaria y autoinmunidad participaron en el Simposio Keystone ‘Tolerancia, Autoinmunidad y Regulación Inmunológica’ en Breckenridge, Colorado, entre el 21 y el 26 de marzo de 2006. Las ponencias describieron los avances en la investigación en el campo de la tolerancia y la autoinmunidad. En este resumen se ha organizado la información en cinco áreas: 1) Genes y señalización en la regulación del sistema inmunológico, 2) Desencadenantes extrínsecos y aspectos intrínsecos de la autoinmunidad, 3) Supresión y regulación del sistema inmunológico, 4) Ensayos clínicos de inmunotolerancia, 5) Debate y reconocimientos a la trayectoria científica.

ABSTRACT
The most relevant international groups working in immunological tolerance and autoimmunity participated in the Keystone Symposia ‘Tolerance, Autoimmunity and Immune Regulation’ in Breckenridge, Colorado, between the 21st and 26th of March 2006. Lectures and abstracts described new research work and advances relevant to tolerance and autoimmunity. In this report, the information is organized in five areas: 1) Genes and signals in immune regulation, 2) Extrinsic triggers and intrinsic drivers of autoimmunity, 3) Suppression and regulation of the immune system, 4) Translating tolerance to the clinic, 5) Debate and awards. The results presented in the meeting demonstrate the continuous advances in the knowledge of tolerance and prove the need to pursue research on this field. This may help to develop clinical trials to prevent autoimmune diseases in at risk subjects.

PALABRAS CLAVE: Tolerancia / Autoinmunidad / Regulación Inmunológica.
INTRODUCTION

Keystone Symposia is a non-profit organization created in 1972 as a communication channel for scientists and the benefit of society. In 1997, the Keystone Symposia became an independent organization based in Silverstone, Colorado. At present, they organize scientific conferences in relaxing locations-over 40 per year with an average of 250 participants per meeting-on biomedical and life science topics that catalyze information exchange and networking (www.keystonesymposia.org).

Self versus non-self discrimination is one of the basic areas of immunology. The loss of self-tolerance often leads to autoimmune disease. The pathways by which tolerance is established and maintained are complex but recent work has provided greater insight into these relevant processes. Understanding how tolerance is generated and broken is critical for the development of future immunotherapy in autoimmune diseases such as multiple sclerosis (MS) and type 1 diabetes (T1D). Novel approaches need to be developed for the treatment of autoimmunity, connecting basic, pre-clinical and clinical studies.

The sessions from the last Keystone Symposia in Colorado, included studies of the genes and the signals involved in tolerance and autoimmunity, the interaction of innate and specific immunity as well as novel approaches for the induction of tolerance in experimental models and their translation to human clinical trials.

GENES AND SIGNALS IN IMMUNE REGULATION

The conferences in this session referred to several molecules involved in the regulation of immune responses and its impact on immunopathology. Dr. A. Rao (USA) pointed out the importance of the cooperation between FoxP3, a transcription factor of regulatory T cells and NFAT in T regulatory cell function. NFAT is a transcription factor that regulates the expression of cytokines and other genes related to the immune system, influencing the T cell state and its activity. The cooperative binding of NFAT and FoxP3 could promote tolerance to T1D in the BDC2.5 NOD mouse model.

Dr. K. Hogquist (USA) talked about genetic changes underlying positive selection. His group identified genes upregulated after positive selection in murine T cells. One of these genes is Kruppel-like factor 2 (KLF2), a transcription factor from the Kruppel-like family, expressed in naïve resting T cells. The Kruppel-like factors constitute a multigene family of transcription factors that have discrete patterns of expression, which implies that they play important biological roles in the tissues in which they are expressed. KLF2 is critical for T cell survival and quiescence, and regulates several genes related to T cell migration (CCR7, CD62L and CD69) but does not affect genes related to survival (BCL2). T cells from KLF2 deficient mice cannot emigrate from the thymus. Therefore, these T lymphocytes are unable to access to survival factors present in lymph nodes.

Dr. D.A. Vignali’s group (USA) centred their efforts in determining the TCR strength required for tolerance induction and function. The aim of the study was to define the requirements of ITAMs, in terms of quality and quantity. The experimental model used was the CD3 knock out mice reconstituted by retrovirus-mediated stem cell gene transfers with CD3ε,γ,δ -with a different number of ITAMs-. The lack of complete signalling caused a low proliferation of T cells, but despite that, these cells produced great amounts of IFNγ. Moreover, when T cells presented only 2 to 6 functional ITAMs, mice developed an autoimmune-like phenotype.

The function of Aire (Autoimmune Regulator) in the thymus has been extensively studied. However, Aire is also expressed in monocyte-derived antigen presenting cells in peripheral lymphoid organs. Dr. S. Hassler (Sweden) showed the first report on the function of Aire in dendritic cells (DCs) and peripheral tolerance: DCs from Aire knock out mice activate naïve T cells more efficiently than normal DCs. Sixty eight genes were up regulated in Aire -/- DCs, such as VCAM1. VCAM1 was also hyperexpressed in monocytes from patients with Autoimmune Polyendocrinopathy Syndrome (APS1). APS1 is a monogenic autoimmune syndrome caused by a defect in the Aire gene. Aire knock out mice displayed an increased number of APCs in blood, lymph node and spleen, correlating to the increased number of monocytes found in peripheral blood from APS1 patients. These data suggest that Aire could regulate DCs homeostasis and tolerance in the periphery.

Two communications were related to the recently discovered Tim family of genes. The T cell immunoglobulin mucin (TIM) proteins are type I membrane glycoproteins expressed on T lymphocytes that regulate the function of T helper cells. Dr. D. Umetsu (USA) determined a possible role of TIM1 in allergic diseases, in the context of the hygiene hypothesis. Both genetic and environmental factors have been implicated in atopic disorders. TIM1 is a polymorphic molecule mainly expressed in Th2 cells. TIM-1 ligand is TIM-4, but TIM-1 is also a receptor for hepatitis A virus (HAV). The prevalence of asthma in HAV+ patients is lower than in non-infected subjects. Polymorphisms in TIM-1 are associated with the risk of Th2 mediated allergic asthma. Interestingly, Dr. Umetsu’s group found an association between TIM1 polymorphism and atopy protection in HAV+ individuals, thus linking genetics and environment.
In 2005, Dr. V.K. Kuchroo (USA) identified a marker for differentiated Th1 cells - TIM-3- and its ligand - galectin-9-. Its interaction with TIM-3 negatively regulates Th1 immunity, leading to an inhibitory signal probably to promote the termination of the Th1 response. He also mentioned more recent data: IL-17-producing CD4+ T cells and their potential role in autoimmunity, as pro-inflammatory mediators. These «Th-17» cells have been recently described as a new CD4+ T subset, independent from Th1 and Th2 lineages(2). He focused on CD4-IL-17 T cell differentiation factors. Interestingly, TGF-β - a Treg inducing factor- can also promote a CD4-IL-17 T cell differentiation in the presence of IL-6. Thus, he proposed a dichotomy in Treg and CD4-IL-17 T cell differentiation, influenced by the local environment.

**EXTRINSIC TRIGGERS AND INTRINSIC DRIVERS OF AUTOIMMUNITY**

The treatment with anti-CD3 antibodies was previously reported to reverse T1D in NOD mice and now it is being tested in human clinical trials, as shown below. Dr. M.G. von Herrath (USA) presented the results of a novel combinatorial treatment for recent-onset T1D in NOD and RIP-LCMV mice models. Von Herrath’s group combined anti-CD3 antibodies with intranasal /oral insulin and proinsulin. They clearly demonstrated an increase in: 1) T1D reversal 2) specific Treg numbers and 3) production of suppressor cytokines by Tregs(3).

In the last years, Dr. P. Ohashi (Canada) has been working in apoptosis related to autoimmunity using experimental models of T1D. To determine whether the apoptosis in beta cells would promote an autoimmune or a tolerogenic response, his group generated the RIP-iCasp3-4 model, a model of caspase-dependent inducible beta cell apoptosis. In these mice, following the controlled apoptosis, an activation of antigen-specific T cells (against beta cells) was described.

According to the hygiene hypothesis, Dr. A. Cook (United Kingdom) analyzed the impact of vaccination strategies and improved hygiene conditions in the lower risk of infections in developed countries. This fact could have consequences in immune system and autoimmunity. She focused on bacterial (Salmonella) and helmint (Schistosoma) infections, and their protective effect to T1D in NOD mice. The prevention after infection could be explained by the mechanisms of antigen presentation, as demonstrated by DC-exposed transfer experiments. Also, an alteration of chemokine profile was proposed as a possible mechanism of protection.

Moreover, Dr. D.J. Cua (USA), who is working in the field of cytokine biology, analyzed the role of IL-23 (a new member of the IL-12 family of regulatory cytokines produced by activated macrophages and dendritic cells) in an experimental model of spontaneous colitis, the knock out mice for IL-10. IL-23 is involved in the pathogenesis of other autoimmune diseases such as inflammatory bowel disease, Experimental Autoimmune Encephalomyelitis (EAE), collagen-induced arthritis, etc. By developing double knock out mice for IL-10 and IL-23 he showed that IL-23 could drive the target organ inflammation in organ-specific autoimmunity.

**SUPPRESSION AND REGULATION OF THE IMMUNE RESPONSE**

Several diseases served as models for the study of regulation of the immune response by invariant Natural Killer T cells (iNKT) and Treg cells: T1D, autoimmune uveitis, rheumatoid arthritis and cancer. Prof. T.L. Delovitch (Canada) described the role of iNKT cells as regulators of T1D in NOD mice. The iNKT cells regulate immune responses, express NK cell markers and an invariant TCR and recognize lipid antigens in a CD1d-restricted manner. He reported the protective effect of these cells in diabetes driven by CD4’CD25’Treg cells. He also observed that alpha-GalCer –an activator of iNKT T cells- caused a similar protective effect, modulated by IL-16 and CCL4. A possible therapeutic effect for these cytokines in diabetes was proposed.

Dr. R.R. Caspi (USA) presented an experimental model of autoimmune uveitis (EAU), inducible by the immunization with retinal Ag (IRBP) in CFA. The IL-1 pathway was necessary to generate a T cell pathogenic response. Moreover, in knock out mice for IRBP treated with IRBP in CFA, she described the generation of Tregs non-specific for retinal antigen(4). To demonstrate the effect of microbial agents in this process, incomplete Freund’s adjuvant (IFA, lacking mycobacterium) was used and Treg activity was not detected. Thus, microbial components appear to activate Tregs of other specificities to inhibit the generation of autoreactive T cells. Non-specific Tregs activated by bystander effects could inhibit autoimmunity. These findings provide the first evidence that generation of specific Treg cells to a native autoantigen in a mouse with a diverse T cell repertoire requires a cognate interaction.

Dr. S. Sakaguchi (Japan) described the SKG mice, a model of rheumatoid arthritis (RA), caused by a mutation in ZAP70 gene. The consequence is an abnormal thymic positive selection in NODskg/skg mice. This affects the generation of diabetogenic T cells but also of Treg, which display a reduced suppressive activity when transferred to nude mice. RA in this model would be dependent on the
abnormal thymic selection of CD4+ T cells, their altered regulation by Tregs and putative environmental factors.

Treg lymphocytes could be determinant in preventing the rejection after transplantation by facilitating specific unresponsiveness. Dr. K.J. Wood (United Kingdom) designed an in vitro approach in which Tregs could control alloantigen rejection, showing that an increase in IFN-γ produced by Tregs supported their suppressive activity to autoreactive cells.

TRANSLATING TOLERANCE TO THE CLINIC

Different tolerogenic protocols with possible clinical application were presented. Dr. D.W. Scott (USA) had designed an experimental therapy mediated by B cells -as APCs- expressing an IgG-antigen fusion construct. This therapy was successful in several autoimmune disease experimental models (EAE, T1D). He presented a murine model of haemophilia (factor VIII K.O. mice), because some patients receiving factor VIII as treatment generated inhibitory autoantibodies to this molecule. Results showed a reduction of humoral response to factor VIII. Based on preliminary results, he also suggested an interaction between tolerogenic B cells and Tregs, via B7-CTLA4.

Dr. L. Chatenoud (France) showed recent human clinical trials for the prevention of T1D. One of the most successful trials was the treatment of T1D patients with non-mitogenic monoclonal antibodies to CD3(5): patients displayed a long-term increase in C-peptide release in response to glucose. The best results were observed in patients with a higher initial secretory response and a recent onset of the disease. Antibodies to CD3 were injected in NOD mice to better understand the mechanisms involved. A clearance in insulitis and an increase in Treg cells in lymph nodes were found. A role for TGF-β, increased in several organs from NOD mice, was proposed in this model because antibodies anti-TGF-β could inhibit the anti-CD3 remission in vivo.

Tregs were also the subject of some tolerogenic strategies. Dr. C. Baecher-Allan (USA) distinguished two different subsets of CD4+CD25(high) T reg cells: DR+ and DR- cells(6). Both subsets were FoxP3+, but DR+ cells expressed the highest level of FoxP3. DR+ cells seemed to need cell-cell contact to suppression. By contrast, DR- cells could also suppress activity by IL-10 production. The differences observed between DR+ and DR- CD4+CD25(high) Treg cells might have implications in their function.

Tregs could be classified in: natural Tregs, CD4+CD25+, which were generated in the thymus and constitutively express FoxP3 and in Tr1, induced in the periphery in response to IL-10. Dr. M.G. Roncarolo (Italy) developed several immunoregulatory protocols, combining the effect of both subsets. One of them was the ex vivo generation of Tr1 cells by IL-10-treated-DCs. On the other hand, they were able to expand CD4+CD25+ ex vivo by rapamicin. A proposed in vivo therapy was the combined administration of rapamicin + IL-10: that could prevent T1D in NOD mice more efficiently than rapamicin alone (their previous in vivo approach). An increase in CD4+CD25+ cells and Tr1 cells in pancreatic lymph nodes and in the islets were detected. This therapy (rapamicin and IL-10) induced a CD4+CD25+ and Tr1 tolerogenic response. This immunointervention has been assessed in islet transplantation tolerance(7).

DEBATES AND AWARDS

During the 2nd day there was a debate between Dr. D. Green (USA), Dr. P. Matzinger (USA), Dr. E. Raz (USA), Dr. N. Greenspan (USA) and Prof. R. Zinkernagel (Switzerland) entitled ‘Friendly or dangerous signals?’ Since immunologists often lack a general vision of immune system, the requirement of a new point of view integrating concepts was suggested, taking into account the overlapping pathways. In the field of autoimmune diseases, R. Zinkernagel minimized the importance of self-non-self discrimination as a key factor in autoimmunity: he hypothesized that the location, timing and expression level of an antigen, are determinant factors in autoimmune response. P. Matzinger stated that alarm systems in injured tissue play a much more important role in immune activation than the antigenic presentation in lymph nodes. The importance of innate immunity was also discussed in organ-specific autoimmune diseases: an stressed or injured tissue, showed a «canonical program» or altered expression profile, tissue specific, including Toll-like receptors, cytokines and chemokines. Thus, the organ-specific features of innate immunity could modulate the adaptive response, depending on the location.

At the end of the meeting, the Keystone Symposia awarded Professor Eli E. Sercarz (Torrey Pines Institute for Molecular Studies, San Diego, CA) and Professor Noel R. Rose (Director of the Centre for Autoimmune Disease Research of the Johns Hopkins University). Prof. Eli E. Sercarz discovered that EAE is caused by a specific group of T cells that drive the disease. By investigating these «driver» T lymphocytes, Dr. Sercarz hopes to find the reason why these specific cells induce inflammation and have such a harmful effect on the brain and spinal cord. He expects to use these autoreactive T cells to develop a vaccine that might stimulate the regulatory cells and treat humans with new vaccination strategies. T1D is another field of research of Prof. Sercarz: his data suggest that unique and/or altered
processing of self antigens may play an essential role in the development and expansion of autoreactive T cells. Professor Noel R. Rose is a pioneer in the field of autoimmunity research. His studies on autoimmune thyroiditis in the 1950s helped to initiate the new research on autoimmune disease. He and his colleagues have continued to contribute to our understanding of autoimmunity, including the first demonstration of the genetic factors responsible for predisposition to autoimmune disease in animals and more recent investigations on the influence of infection and environmental agents in the initiation of autoimmune disease in genetically predisposed animals. He is co-author, together with Prof. I. Mackey, of the textbook ‘The Autoimmune Diseases’, the first book that considered autoimmune diseases collectively. His research lines include studies on autoantigenic epitopes of thyroglobulin, the mechanisms of virus-induced myocarditis, and the mechanism by which certain drugs can cause autoimmune hepatitis.

In summary, the data presented in the symposia showed the most recently published and even unpublished data in the field of autoimmunity and tolerance. The current clinical trials for the prevention and reversal of autoimmunity are encouraging and demonstrate that basic knowledge in immunology contributes to the design of new immunotherapies.

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