Cytokines and Chemokines in physiopathological processes.
IX Monographic Symposium of the Spanish Society for Immunology

V. Mas-Bosch¹, C. González², M. Rodríguez¹, N. Amézaga¹, J. Calvo¹, A. Gayà³

¹Servei d’Immunologia, Hospital Clinic, Barcelona. ²Servei d’Immunologia, Hospital de la Santa Creu i Sant Pau, Barcelona. ³Fundació Banc de Sang i Teixits de les Illes Balears, Palma de Mallorca.

CITOCINAS Y QUIMIOCINAS EN PROCESOS FISIOPATOLÓGICOS.
IX JORNADAS MONOGRÁFICAS DE LA SOCIEDAD ESPAÑOLA DE INMUNOLOGÍA

Recibido: 9 Diciembre 2004
Aceptado: 17 Diciembre 2004

The IX Monographic Symposium of the Spanish Society for Immunology (S.E.I.) was held in Palma de Mallorca on the 15th and 16th October. It was focused on the role of cytokines and chemokines in physiopathological processes. Doctors Antoni Gayà and Javier Calvo from the Fundació Banc de Sang i Teixits de les Illes Balears (Palma de Mallorca) organised the Meeting with the support of «SA NOSTRA», Col·legi Oficial de Biòlegs, Fundació Mateu Orfila and Govern de les Illes Balears. During the Opening Ceremony, Dr. Antoni Gayà and the former president of the S.E.I, Dr. Jordi Vives, gave introductory speeches.

The first part of the Symposium was devoted to the physiology of cytokines, chemokines and their receptors. This part was composed of three speeches and Dr. R. Alvarez, from H. Virgen de la Arrixaca (Murcia), moderated it. Dr. Carmen Gutiérrez from H. Central de Asturias (Oviedo), in the first place, gave a general vision of cytokines from their discovery twenty years ago until present times. She focused on the different sets of cytokines released by Th1 and Th2 lymphocytes, as well as on those cytokines showing pro- (TNFα, IL-6…) and anti-inflammatory effects. Dr. Manuel Juan from Hospital Universitari Germans Trias i Pujol (Badalona) first defined the concept of chemokines as chemotactic cytokines. He then described their function and taxonomy. Finally, he referred to his work on CCL4 polymorphisms due to alternative splicing sites. The two speeches were very useful for understanding the rest of the talks properly. Dr. José Zamorano, from Hospital San Pedro de Alcántara (Cáceres) closed this first part. He presented his work on the molecular activation of STAT-6 by Interleukin 4 (IL-4), a cytokine belonging to the haematopoietin family. IL-4 is mainly expressed in T lymphocytes and mast cells and it shows important anti-apoptotic activities. IL-4 activates the transcription factor STAT-6, which has been involved in asthma in humans. Dr. Zamorano highlighted the fact that salicilates inhibit IL-4 responses by decreasing STAT-6 levels. This activity could help improve the course of asthma in humans.

The next part of the Symposium consisted of two speeches and was also moderated by Dr. R. Alvarez. Dr. José A. Andrades from Universidad de Málaga (Málaga) reviewed his experience on recombinant growth factors and cellular capacitation for regenerative medicine. Firstly, he explained the obtention of a hybrid growth factor composed by basic fibroblast growth factor (bFGF) (with mytogenic and wound healing functions), and a collagen binding domain. Dressings embeded with this hybrid growth factor showed an acceleration of wound healing in rats and humans. He also referred to his work on amplification and selection of condro and osteo progenitor cells and their posterior induction to produce cartilage and bone, respectively. This method was successfully used in bone fracture wound healing of both, rats and humans. Dr. Jaume Pons, from Hospital Son Dureta (Palma de Mallorca), held the last speech of this block. He
reviewed the importance of mutations in genes coding for cytokines and their receptors as the source of primary immune deficiencies. He started by highlighting that mutations in the common γ (γc) chain of several cytokine receptors, as well as in Jak 3 and IL-7Rα, led to severe combined immunodeficiency (SCID). After that, he focused on the molecular basis of phagocyte deficiencies, which makes the host susceptible to mycobacteria and salmonelae infections. Mutations in one of the following genes: IL-12, IL-12 receptor β1 chain, or either of the two protein subunits, R1 and R2, of the IFN-γ receptor are responsible for this immune deficiency.

After that, the main Conference entitled «Cytokines type I and II: Innate and Acquired Immunity regulation» was presented by Dr. Manuel Fresno from the Centro de Biología Molecular (Madrid). Dr. Fresno concentrated his conference on the concept of the Th1/Th2 paradigm. First of all, he explained that an immature effector T cell (Th0) could differentiate either to a Th1 cell, under the effect of IFN-γ and IL-12, or to a Th2 one, when IL-4, IL-5 or IL-6 are elicited. Th1 cells lead to cell-mediated immunity, whereas the predominant production of Th2 cells provides humoral immunity. IL-4 can inhibit Th1 whereas IFN-γ is able to inhibit Th2 cells, leading to a protection/pathology dichotomy. In intracellular infections with Trypanosoma cruzi a Th2 response not only doesn’t have a protective effect, but it even makes the course of the infection worse. In experiments with fungus, however, it has been established that the Th1/Th2 ratio in direction Th1 was critical to avoid disease.

Dr. Fresno reviewed the main differences between innate and adaptive immunity and introduced the concept of Pathogen-Associated Molecular Patterns (PAMPs). Interestingly, PAMPs on the surface of intracellular pathogens would be presented by dendritic cells (DC) expressing IL-12, leading to a Th1 cell response. In contrast, extracellular pathogens would be presented by specific DC releasing IL-4, thus leading to a humoral response. In summary, specific pathogens would be recognized by specific DC, which would release specific cytokines, which would lead either to Th1 or Th2 cell differentiation. Finally, it must be mentioned that some of the factors influencing the vaccine response are the nature of antigen and its dose, the Th1/Th2 ratio, and the type of cytokines released.

The therapeutic use of cytokines was the next item to be discussed. It consisted of three speeches and was moderated by Dr. Francisco Lozano from Hospital Clínico de Barcelona. Dr. Juan Gómez-Reino from Hospital Clínico de Santiago de Compostela (La Coruña) dealt with the topic of cytokine antagonists and their clinical application. TNF-α is a pro-inflammatory cytokine, which plays an important role in several immune-mediated inflammatory disorders, such as rheumatoid arthritis, spondilitis or psoriasis. TNF-α antagonists, such as infliximab or etanercept are used in the treatment of these malignancies. Nevertheless, bystander effects have been reported in 5% to 7% of the patients. Reactivation of tuberculosis, including miliar and extrapulmonar variants, is one of the worst adverse side effects observed. Dr. Mario Mellado from the Centro Nacional de Biotecnología (Madrid) was the next speaker. He presented his work on dimerisation of chemokine receptors as new targets for therapeutic intervention. Chemokine receptors not only bind chemokines, but some of them also serve as co-receptors for HIV infection. Chemokine receptors can be found as monomers and dimers; the monomeric state allows activation and regulation of associated proteins. Dr. Mellado and his group have identified two amino acids, Ile52 and Val150, that are crucial in CCR5 dimerisation, thus defining possible targets for therapeutic intervention in chemokine related diseases.

Dr. Ignacio Melero from Universidad de Navarra (Pamplona) discussed on antitumoral immunotherapy strategies based on chemokine and cytokine gene transfer. IL-12 is a cytokine, which inhibits angiogenesis and induces IFN-γ synthesis. Dr. Melero focused on the use of IL-2 plus IL-12 combined gene therapy in s.c. tumor nodules derived from the CT26 murine colorectal adenocarcinoma cell line. IL-10 and IL-12 synergize, reaching 100% of tumour eradication. Moreover, he presented data on DC transfected with a vector encoding the human IL-12 gene and, after that, injected them directly in the tumour. There, the DC are loaded with specific tumour antigens and then migrate to the draining lymph node, where they encounter naïve CD4+ T-cells. In presence of IL-12 and IFN-γ CD4+ T-cells are committed to differentiate into Th1 cells. This local gene therapy emerges as a promising strategy.

The next topic of discussion was autoimmune pathology, also moderated by Dr. Francisco Lozano. Dr. Javier Martín from Instituto de Parasitología y Biomedicina López-Neyra (Granada) dealt with the influence of cytokine gene polymorphism in the predisposition/severity of rhematoid arthritis (RA). He investigated the possible association between the IL-6 promoter polymorphisms, at positions -622 and -714, and susceptibility to, and/or outcome of RA. No difference was observed in the distribution of IL-6 promoter genotype or allele frequencies between RA patients and controls. However, a significant difference in the mean age at disease onset between IL-6 genotypes was observed. No other associations were found by analysing the TNF-α, IL-2, or IL-12 promoters. Dr. Antonio Núñez from Hospital Virgen del Rocío (Sevilla) talked about chemokine
polymorphisms in autoimmunity. He reviewed his work on MCP-1 promoter polymorphisms in Spanish patients with RA. No association between -2518 (A/G) MCP-1 polymorphism and susceptibility to RA was found. Nevertheless, when patients and controls were stratified according to their HLA (HLA-DRBI*) shared epitope (SE) status, a significant increase in the frequency of the GG genotype was found among SE negative (SE-) patients with respect to both SE positive (SE+) patients and SE controls. Dr. Núñez also presented another study, regarding the possible association of CCR2 and CCR5 chemokine receptor gene polymorphisms 190 A/G and D32, respectively, with the susceptibility to systemic lupus erythematosus (SLE). Polymorphisms of CCR2 and CCR5 do not seem to be involved in susceptibility to SLE, although a slight contribution of the CCR5 polymorphism in the production of anti-dsDNA autoantibodies in the development of lupus nephritis, and in the outcome of the disease could be postulated.

The last block of the day was devoted to cytokines and infection and it was moderated by Dr. J. Vives from Hospital Clínic (Barcelona). The first of the three speeches concerning this topic was held by Dr. Pere Joan Cardona from Hospital Universitari Germans Trias i Pujol (Badalona), who talked about the importance of the balance of the Th1-Th2 response in the control of M. tuberculosis infection. Dr. Cardona highlighted the existing confusion towards the topic of tuberculosis’ chronicity. The latent form of tuberculosis could be justified by the existence of a Th1-Th2 balance. Th1 cells are activated by macrophages containing phagocytosed bacilli. A Th2 response would be activated by extracellular bacilli debris. These Th2 cells would release a set of cytokines, which would suppress a Th1 response, thus justifying the chronicity of the infection. On the contrary, other groups support the evidence that a Th2 response doesn’t antagonize with a Th1 one in mice. There is evidence that Th1/Th2 dichotomy is not valid in humans. He also wanted to make us aware of the important role of the bacterium itself, as a living organism, in the generation of chronic infection. He reviewed the fact that his colleagues from Hospital Universitari Germans Trias i Pujol have developed a vaccine called RUTI, that could be useful in reducing the period of isoniazide intake from nine to only two months. Dr. José A. Esté, also from Hospital Universitari Germans Trias i Pujol (Badalona) was the next speaker. Dr. Esté reviewed the role of chemokine receptors as therapeutic targets against HIV infection. In fact, drugs currently used against HIV, such as protease inhibitors or antiretrovirals, are very useful but show important side effects. So, there is a need for finding out different therapeutic strategies. It has been shown that individuals with CCR5 chemokine receptor mutations remain protected towards HIV infection. Therefore, a drug targeting this receptor could block the entry of the virus in the cell. Nevertheless, it should be taken into account that they interfere with a host molecule, which could have negative consequences for the organism. Moreover, the virus could make a co-receptor switch and use CXCR4 to infect the cell. To avoid this problem, drugs inhibiting both CXCR4 and CCR5 chemokine receptors should be used, in combination with other drugs. Viral genes that modulate the immune response and apoptosis was the topic of the last speech of the day. It was given by Dr. Yolanda Revilla from the Centro de Biología Molecular (Madrid). Dr. Revilla first referred to the ability of African swine virus to control apoptosis of the infected cell. This virus stops apoptosis and, once its replication is finished, allows its reactivation. Her group is studying the responsible mechanisms at the moment. Finally, she described how A238L, an African swine virus protein, inhibits cyclooxygenase 2 (COX2) gene expression. COX2 expression is regulated by the transcription factor nuclear factor of activated cells (NFAT). In resting cells phosphorylated NFAT localizes in the cytoplasm; upon stimulation it is dephosphorylated by calcineurin and translocates to the nucleus, where it becomes transcriptionally active. A238L is able to inhibit NFAT activation by inhibiting calcineurin phosphatase activity. As a consequence, COX2 gene expression is inhibited, thus leading to an absence of IL-2, which in turn diminishes proliferation.

On Saturday the 16th the only block of sessions was on inflammatory pathology. Dr. Miguel López-Botet from the Universitat Pompeu Fabra (Barcelona), moderated it. Dr. Álvaro Merino, from Instituto Cardiológico de Clínica Rotger (Palma de Mallorca), presented a study, aimed to demonstrate the interaction between thrombosis and inflammation. His group has used angioplasty as a model of acute coronary syndromes with plaque rupture. Half of the patients received a selective inhibitor of the glycoprotein IIb/IIIa platelet receptor and the other half was given standard antithrombotic therapy. The inflammation markers studied were the serum levels of C reactive protein (CRP), IL-6, IL-1β, IL-8, IL-10, TNF-α and the IL-6 gene promoter –174 GC polymorphism. The study showed a relation between an acute thrombotic condition and increases in CRP and IL-6 levels. The next speech was held by Dr. Javier Calvo from the Fundación Banc de Sang i Teixits de les Illes Balears (Palma de Mallorca). Dr. Calvo first described IL-6 functions and then referred to the –174GC polymorphism. He pinpointed that GG genotypes are related to high IL-6 levels, whereas CC express low IL-6 levels. Dr. Calvo explained that this polymorphism plays a significant role in a variety of diseases, such as Alzheimer disease or...
atherosclerosis. He analysed the genes encoding for HLA, IL-1, IL-6, IL-10, IL-12, IFN-γ and the -174GC IL-6 promoter polymorphism in patients infected with the hepatitis C virus (HCV) to establish their possible relationship with chronicity and therapeutic response. He observed that the frequency of the -174 GG genotype was higher (63%) in HCV infected people than in controls (44%) and concluded that high IL-6 levels may act inhibiting a proper Th1 response. The last speaker of this part was Dr. Jaume Martorell from Hospital Clínic de Barcelona (Barcelona). He divided his speech into four sections. First he referred to cytokines and alloimmune response in the absence of immunosuppressive drugs. After that, he focused on cytokines and immunosuppressive drugs and concluded that calcineurin activity was powerfully inhibited in those patients receiving Cyclosporin A and FK506 (Tacrolimus) at usual doses, excluding underdosification, as the cause of rejection. In the third part of his speech, he talked about cytokines and induction of allo-tolerance. He focused on his work with mesenchimal cells, which present donor's antigens and at the same time have an immunosupressive effect, probably due to IL-10 release. Finally, he devoted the last part of his speech to new perspectives in cytokines and transplantation.

The afternoon session consisted of two main conferences. The first of them was held by Dr. Martínez-Alonso from Centro Nacional de Biotecnología (Madrid). The topic of interest was the role of chemokines in cancer. Dr. Martínez-Alonso first started by defining the concept of chemotaxis (directed migration of leukocytes towards a chemoattractant gradient) and how chemokines induce the polarisation of T-lymphocytes, with generation of specialised cell compartments. Chemokine receptors concentrate at the leading edge of the cell and their function is to sense the direction of migration. Adhesion molecules redistribute to the rear pole of the lymphocyte, the uropod and play a role in the recruitment of bystander leukocytes. After that, Dr. Martínez-Alonso focused on his work about a potential immune escape mechanism by melanoma cells through the activation of chemokine-induced T cell apoptosis.

Dr. Francisco Sánchez-Madrid from Hospital de la Princesa (Madrid) was the last speaker of the Symposium. The title of his speech was «Physiological role of activation molecule CD69 in inflammatory immune response regulation». CD69 is a C-type lectin, and is one of the earliest specific antigens acquired during lymphoid activation. Dr. Sánchez-Madrid exposed his investigations on the susceptibility of CD69-/- mice to tumours. He showed that CD69-/- mice present a great immune response against tumour cells, supporting a novel role for CD69 as a negative regulator of the immune response. CD 69 increases TGF-β production, leading to inhibition of both cell differentiation and proliferation. Mice lacking CD69 present lower levels of TGF-β, increased MCP-1 chemokine production and decreased lymphocyte apoptosis. Finally, he focused on another topic, the role of CD69 in autoimmune reactivity. He analysed a model of collagen-induced arthritis (CIA) in wild-type (WT) and CD69-deficient mice. He showed that TGF-β1 and TGF-β2 levels were decreased in inflammatory foci of CD69-/- mice. The specific T and B cell responses against cartilage type II collagen were increased, as well as some proinflammatory cytokines (IL-1β, TNF-α), all these leading to an exacerbated form of CIA.

The closing ceremony was by Dr. M. López-Botet, as current president of the Spanish Society for Immunology, who acknowledged Doctors Antoni Gayà and Javier Calvo for the brilliant organization and highlighted the variety and quality of the works presented.

CORRESPONDENCE TO:
Virginia Mas-Bosch
Servei d’Immunologia
Hospital Clinic, Villarroel 170, 08036 Barcelona
Phone: 34-932275488. Fax: 34-934518038.
E-mail: vmasbosc7@med.ub.edu