Instituto Juan March
de Estudios e Investigaciones
INSTITUTO GULBENKIAN DE CIÊNCIA

152 CENTRO DE REUNIONES INTERNACIONALES SOBRE BIOLOGÍA

Co-sponsored by
EMBO EUROPEAN MOLECULAR BIOLOGY ORGANIZATION

Workshop on

Molecular and Genetic Basis of Autoimmune Diseases: SLE and RA

Organized by

A. Coutinho, W. Haas and C. Martínez-A.

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Introduction

Systemic lupus (SLE) and Rheumatoid Arthritis (RA) are amongst the earliest described autoimmune diseases (AID). They have been extensively studied both at the clinical and basic levels.

AID continue to increase in prevalence in the western world reaching up to 10% of the general population in some countries. For their variety, age of presentation, and chronic/debilitating clinical course, they represent a major socio-economical problem for public health. From the basic point of view, AID raise fundamental questions and offer a wealth of observations on one of the unsolved questions of modern immunology –natural tolerance to body tissues and its relationship to infections. From the medical point of view, we continue to be unable to diagnose AID before lesion of the target organ or function, and have no rational, curative therapies for diseases such as type I diabetes, rheumatoid arthritis, multiple sclerosis and systemic lupus.

In recent years, major progress in understanding the molecular basis of complex processes in cell and tissue biology have been achieved, particularly in the immune system itself and in the physiology of some of the most common target tissues for AID.

Furthermore, the advances in "genomics" have promoted a wealth of studies on the genetic basis of susceptibility/resistance to AID in human populations. Finally, a number of clinical trials have recently been initiated, testing the beneficial effects of a variety of "biology-based" therapies.

The workshop was divided in five sessions, organized from basic immune mechanisms of tolerance and autoimmunity, followed by the genetics of experimental and human SLE and RA, by the analysis of potential therapeutic targets (cytokines, chemokines and their receptors) and by novel therapeutic approaches, that included the analysis of the "biological" therapeutics (monoclonal antibodies and receptor-fusion proteins) as well as the use of stem cell treatment of AID. Therefore in this workshop scientists coming from different disciplines had the opportunity to integrate data from the clinical and therapeutic perspectives with the molecular and cellular processes that form the basis for these diseases.

Session 1: Lymphocyte biology and tolerance
Chair: Werner Haas

Peripheral T cell tolerance: contribution of parenchymal cells

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German Cancer Research Center and University of Heidelberg

The immune system is an adaptive defence system capable of specifically recognizing and eliminating an apparently limitless variety of foreign invaders. The key challenge of such defence system is to have as broad a T and B cell repertoire as possible in the absence of autoreactivity. There is general support for the concept that professional antigen presenting cells (APC) such as dendritic cells can stimulate as well as turn off T cell reactivity, although the molecular basis for the different results is missing. It is, however, debated whether or not non-professional APC can also contribute to T cell tolerance. Therefore, the role of parenchymal cells in the development and maintenance of self-protection will be discussed.

In order to understand the conditions leading to either activation or silencing of mature T lymphocytes we established some time ago T cell receptor (TCR) transgenic mice specific for the major histocompatibility (MHC) class I antigen K^b (Des-TCR). Most CD8 T cells in these transgenic animals express this particular K^b-specific TCR and can be identified by anti-clonotypic antibodies (Desire-1). Additional expression of K^b in various extrathymic tissues illustrated that the immune system can by-pass undesirable reactivity of mature peripheral T cells by employing various forms of deletional or non-deletional tolerance.

However, the mechanisms remained unclear, because naive T cells have a restricted migration pattern in the adult. How could we then explain tolerance to K^b expressed, for example, only on keratinocytes (2.4KerIV-K^b mice)? We found that neonatal skin, but not adult skin, was accessible for naive CD8 T cells, allowing T cells direct contact with keratinocytes. This interaction led to K^b-specific tolerance (1). It was now of interest which mechanism could prevent naive T cells continuously leaving the thymus in adult life, from rejecting K^b-positive grafts. To evoke dominant tolerance processes in our system would require that T cells, which had been rendered tolerant to K^b in the neonatal phase, persist indefinitely and interfere with the activation of naive, K^b-specific T cells when confronted with a K^b-positive graft. The number of these tolerant cells could be enriched within the $CD8^+$, Des-TCR^+ T cell pool by removing the thymus of Des-TCRx2.4KerIV-K^b animals at 2 weeks of age and, thereby, preventing thymic T cell export during adulthood when naive T cells are not tolerized any longer to K^b on keratinocytes. We now tested whether the tolerant T cells could influence the reactivity of naive T cells. T cells from Des-TCR.RAG-$2^{-/-}$ mice could reject P815-K^b.B7 tumor grafts after transfer into day 15 thymectomized 2.4KerIV-K^b.RAG-$2^{-/-}$ animals transgenic for anti-Leishmania TCR (not crossreacting with K^b). In contrast, day 15 thymectomized Des-TCRx2.4KerIV-K^b.RAG-$2^{-/-}$ mice, which had received

the same number of naive $CD8^+$, Des-TCR T cells, accepted the respective tumor graft. Thus, CD8 T cells with a given TCR can either develop into destructive effector cells or into tolerant "regulator" cells, depending on the conditions of antigen encounter. TGFβ seems to play an important role in this type of tolerance as indicated by comparison of gene expression of tolerant and activated/naive T cells using micro array technology and quantitative RT-PCR. Expression of TGFβ as well as of 3 TGFβ-induced proteins was more than 4-fold upregulated in tolerant cells.

Additional evidence of the capacity of non-professional APC to induce T cell tolerance was obtained from studies of liver sinusoidal endothelial cells (LSEC). These organ-resident, non-myeloid antigen-presenting cells are capable of cross-presenting soluble exogenous antigen to $CD8^+$ T cells. While LSEC employ similar molecular mechanisms for cross-presentation as dendritic cells, the outcome of cross-presentation by LSEC is $CD8^+$ T cell tolerance rather than immunity. As uptake of orally applied antigens into LSEC occurs efficiently *in vivo*, it is likely that cross-presentation by LSEC contributes to oral tolerance (2).

References:

1. Alferink et al Alferink, J., Aigner, S., Reibke, R., Hämmerling, G. and Arnold, B. Peripheral T cell tolerance: the contribution of permissive T cell migration into parenchymal tissues of the neonate, Immunol. Rev. 169: 255-261, 1999
2. Limmer, A., Ohl, J., Kurts, C., Ljunggren, H.-G., Reiss, Y., Momburg, F., Arnold, B., and Knolle, P. 2000. Efficient presentation of exogenous antigen by liver endothelial cells to $CD8^+$ T cells results in antigen-specific T cell tolerance. Nature Medicine, 6: 1348–1354, 2000

Cell migration in inflamed tissue

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Lymphocyte migration into tissue or homing to secondary lymphoid organs occurs through a slow rolling movement in specialized postcapillary vessels, the high endothelial venules (HEV). Rolling is mediated by transient L-selectin bonds with peripheral node addressin (PNAd), a mixture of glycoproteins expressed on HEV. The binding of a chemokine to its receptor induces sequential conformational changes; this leads ultimately to the formation of a signaling complex, the "chemosome", responsible for activation of chemokine-related signaling events. Ligand binding first induces the receptor changes that expose epitopes required for dimerization; this allows access of JAK kinases to the receptor, which are then tyrosine phosphorylated. JAK association to the chemokine receptor, but not its dissociation, takes place in the presence of pertussis toxin (PTx), whereas Gαi does not associate when cells are pretreated with a JAK-specific inhibitor. This indicates JAK-dependent Gαi association to the receptor, and a role for Gαi in fine-tuning the JAK/STAT pathway.

In addition, chemokines trigger an increase in integrin avidity/affinity, promoting integrin-mediated cell adhesion. This creates a hierarchy of signaling events that can be separated kinetically and topologically. One of the earliest detectable biochemical responses to fibronectin (Fn)-induced integrin engagement is the rapid tyrosine phosphorylation of various intracellular proteins. This is due to activation of non-receptor protein tyrosine kinases, the membrane-associated Src family kinases (SFK), the cytoplasmic p125 focal adhesion kinase (FAK) and the phosphatase SHIP-2. Data will be discussed that clearly illustrate the presence of the different components of the "chemosome" in specific cell surface raft microdomains that promote more efficient signaling

Diversity of regulatory T cells controlling the onset of autoimmune diabetes

Jean-François Bach

Progression of insulitis and onset of diabetes in the non-obese diabetic (NOD) mouse is placed under the tight control of CD4+ regulatory T cells. This is best illustrated by disease acceleration observed after thymectomy at weaning. The direct role of CD4+ CD25+ T cells has been demonstrated in a cotransfer model in which such cells were shown to inhibit the diabetogenic potential of T cells derived from diabetic mice transferred into NOD/scid recipients. The regulatory activity is not limited to CD25+ T cells since CD62L+ CD25- T cells can also protect NOD mice from diabetes. Furthermore, the CD25+ compartment is heterogeneous. In addition to activated T cells, one can distinguish $CD25^{high}$ T cells which are potent suppressors *in vitro* in a TGFβ independent manner and $CD25^{low}$ T cells which also suppress the proliferation of CD25- T cells *in vitro* but do it in a TGF β dependent manner. The latter but not the former population is depleted by 3 week thymectomy. Both populations act by cell-cell contact but their mode of action is unclear (CTLA-4, GITR, membranes TGFβ, ...).

Other regulatory T cells may protect NOD mice from diabetes, notably Th2 cells (after administration of soluble βcell autoantigens), NKT cells and CD8γδ Tcells.

It will be important to determine if these various regulatory T cell subsets are related and if so, whether they can be successfully used therapeutically after adequate stimulation.

Regulatory T cells in autoimmune and allergic disease

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Regulatory T cells (Tregs, also referred to as Suppressor T cells) are important components of the homeostasis of the immune system, as impaired regulatory T cell activity can cause autoimmune diseases and atopy. It is now clear that the name "regulatory T cells" encompasses more than one cell type. For instance, CD4+CD25+ regulatory T cells have received attention due to their immunosuppressive properties *in vitro* and *in vivo*, but in several instances it has been shown that CD4+CD25- T cell populations also contain potent regulatory activity. Recent progress in the field of regulatory T cells includes the improved understanding of the role of costimulatory molecules and the cytokines IL-10 and IL-2 in the induction and function of regulatory T cells, and the generation of CD25+ and CD25- regulatory T cells *in vivo* through high-avidity T cell receptor interactions.

Data will be shown on two experimental models in which Tregs play a key role, spontaneous experimental autoimmune encephalomyelitis (EAE) and Hyper IgE responses.

Session 2: Mechanisms of SLE and RA
Chair: Carlos Martínez-A.

B cell homeostasis

Antonio A. Freitas

UBPL, Institut Pasteur, France

Cellular competition for survival signals offers an appealing mechanism for the maintenance of cellular homeostasis. I will present an experimental investigation of the role of competition for resources in the regulation of peripheral B cell numbers.

We used competitive repopulation experiments to show that B cells must compete to persist in the periphery. We studied the ability of a limited number of normal B cell precursors to populate peripheral B cell pools. We found that the physiological number of peripheral B cells is not determined by the number of B cell precursors. Chimeras with a three fold reduced rate of BM B cell production have normal numbers of peripheral B cells. Parabiosis between normal and B cell-deficient mice show that the BM B cell production of one mouse suffices to replenish the B cell pool of three mice. We studied the competitive repopulation by different B cells of irradiated mice reconstituted with bone marrow from either congenic or Ig-transgenic mice mixed at variable ratios. We found that in chimeras hosting TG and non-TG cells at the periphery, non-TG cells are preferentially selected. The selection of non-TG only occurs when population growth plateaus i.e. when resources become limiting and competition starts to operate and that the life-expectancy of the same B cell population differs according to the second population present. The present results show that the life-span and the population size of each B cell clone can be altered (interfered with) by the presence of a second cell population, demonstrating the existence of cellular competition among B cells.

Receptor editing and autoimmunity

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Receptor editing carries out active self-tolerance. The editing process exploits rearrangement signals flanking or inside rearranged V genes to change, inactivate or delete V(D)J genes coding for autoantibodies. The process acts on autoreactive B cells because receptor-(self)antigen interaction sustains rearrangement, allowing the B cell to scan the remainder of the V gene repertoire for examples (i.e. editors) that veto the self reactivity of the first receptor. In the case of anti-DNA B cells the editors are drawn mainly from the V genes of kappa and lambda L-chains. They include V genes coding for V regions that efficiently edit H-chains of anti-DNAs. These V regions are highly acidic and they edit by neutralizing H-chain arginine residues that interact with DNA.

We have analyzed the genetic control of anti-DNA in the Lupus mouse MRL/lpr in the context of this mechanism of self-tolerance. We assumed that the expression of anti-DNA in Lupus might result from a failure to edit. By this model we would expect anti-DNAs with the original L chain. Although the anti-DNAs do include L chains that sustain DNA binding, these B cells show evidence for multiple rearrangement attempts. Therefore, we think that the anti-DNAs arise not from failure to edit but because of sustained rearrangement. Such, overzealous editing would replace editor L chains with L chains that sustain DNA binding. Because the preimmune repertoire has a high frequency of anti-DNA B cells the un-editing process should produce high frequencies of anti-DNAs and can explain why anti-DNA is the most common autoantibody in disease.

B cells in rheumatoid arthritis

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RA is marked by a chronic inflammation of the joints and as the disease progresses, lymphocytes migrate into the synovial membrane. However, the degree of infiltration and lymphocyte composition varies. In some patients inflammatory cytokines seem to support the development of ectopic lymphoid tissue and structures develop that are reminiscent of primary B cell follicles (Randen et al, 1995, Takemura, et al 2001). Just as in the lymphoid organs, B cells home into a network of follicular dendritic cells where antigen activation leads to B cell proliferation and differentiation. To understand the immune processes in the inflamed synoviium, single B cells were directly micro-disseceted from frozen tissue sections and their V-gene repertoire determined (Schröder et al, 1996). The sequence analysis suggested that within the synovial tissue a germinal centre reaction is induced. The V-gene repertoire is diversified by hypermutation and those B cells expressing high affinity receptors are selected to differentiate within the synovial tissue into plasma cells (Kim et al, 1999).

However, in the majority of patients ectopic germinal centres do not develop and yet plasma cells still accumulate in the inflamed synovial tissue (Kim et al, 2000). Since these cells must originate in the lymphoid organs we investigated more closely the B cell subsets in the peripheral blood of patients with RA. FACS analysis showed that in contrast to healthy individuals, RA patients have numerous plasma cells, suggesting that the chronic inflammation leads to a continous activation and differentiation of B cells (Lindenau et al, 2003). Single plasma cells were sorted and RT-PCR was performed to determine the V-gene repertoire. The finding of high numbers of somatic mutations supports the interpretation, that in RA patients B cells are chronically activated.

It is of key interest to determine the antigens that are responsible for this chronic activation. A surprising finding of the group of D. Mathis was that antibodies, specific for self-antigens which are commonly expressed in every cell in the mouse, may induce arthritis (Matsumoto et al, 2002). It is possible that also in human such self antigens exist that stimulate an autoimmune response which induces and / or promotes the development of RA. To search for such antigens a cDNA expression was constructed from testis, a tissue, where due to hypomethylation a broad spectrum of genes, representative of the human gene repertoire, is expressed. This library is used to test for the specificity of the antigen activated B cells in the peripheral blood of RA patients.

References:

Kim, H.-J., Krenn, V., Steinhauser, G. and Berek, C. 1999 Plasma cell development in synovial germinal centers in patients with rheumatoid and reactive arthritis. J. Immunol.162, 3053 - 3062

Kim, H.-J. and Berek, C. 2000. B cells in rheumatoid arthritis. Arth. Res. 2: 126-131

Lindenau, S., Scholze, S., Odendahl, M., Dörner, T., Radbruch, A., Burmester, G.-R. and Berek, C. 2002. Abberant activation of B cells in patients with rheumatoid Arthritis. N.Y. Acad. Scien. in press

Magalhaes, R., Stiehl, P., Morawietz, L., Berek, C. and Krenn, V. 2002. Morphological and Molecular pathology of the B cell response in synovitis of Rheumatoid Arthritis. Virchows Arch. 441: 415 – 427

Matsumoto, I., Maccioni, M., Lee, DM. et al (2002). How antibodies to a ubiquitous cytoplasmic enzyme may provoke joint- specific autoimmune disease. Nat. Immunol. 3: 360-365

Randen, I., Mellbye, O.J., Forre, O., Natvig, J.B. 1995. The identification of germinal centres and follicular dendritic cell networks in rheumatoid synovial tissue. Scand. J. Immunol. 1: 481-486

Schröder, A.E., Greiner, A. Seyfert, C. and Berek, C. 1996. Differentiation of B cells in the non-lymphoid tissue of the synovial membrane of patients with rheumatoid arthritis. Proc. Natl. Acad. Sci. USA 93: 221 – 225

Takemura, S,, Braun, A., Crowson, C., Kurtin, P.J., Cofield, R .H., O'Fallon, W.M., Goronzy, J.J, Weyand, C.M. 2001. Lymphoid neogenesis in rheumatoid synovitis. J. Immunol. 167: 1072-1080.

Stem cell transplantation in severe autoimmune disease - Current status and future directions

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Immunoablation and rescue with autologous hematopoietic stem cell transplantation (HSCT) has been applied to around 600 patients world wide suffering from severe autoimmune disease (AD). The combined EBMT/EULAR data base (107 transplant teams in 21 countries) contains 468 reports, 15 allogeneic (mostly hematological ADs) and the rest autologous.

Of the 449 mobilised patients, 439 proceeded to transplant, the others either improving such that transplant was not undertaken, or dying due to AD or transplant related factors.

Autologous HSCT was performed in the following ADs: multiple sclerosis (MS) n=135, systemic sclerosis (SSc) n=72, rheumatoid arthritis (RA) n=72, juvenile idiopathic arthritis (JIA) n=51, systemic lupus erythematosus (SLE) n=55, dermato/polymyositis /DM/PM) n=7, idiopathic thrombocytopenic purpura (ITP) n=12, pure red cell aplasia (PRCA) n=4, and other rarer disorders in small numbers including myasthenia gravis, Sjoegren's syndrome, ankylosing spondylitis, vasculitis, cryoglobulinaemia, relapsing polychondritis, Evans syndrome, autoimmune haemolytic anaemia and inflammatory bowel disease.

Median follow-up is 20 months (1-81) with an interval diagnosis to HSCT of 6 years (1-28). 65% were females with a median age of 34 (2-69).

All patients were treated within the context of phase I/II pilot studies using a limited number of protocols, as recommended in a consensus statement (1). Most received HSCT from a peripheral blood source and 45 from bone marrow, mostly children with the systemic form of JIA (Stills Disease). Mobilisation was mostly performed with a combination of cyclophosphamide (Cy) 2-4 g/ m^2 and G-CSF with half as many G-CSF alone and a few GM-CSF or other regimens.

The graft was purged with CD34 selection only in 195, CD34 selection plus T-cell depletion in 33 and unmanipulated in 130. The most frequent conditioning regime was Cy 200-150 mg/kg body weight (n=110), followed by Cy plus ATG/ALG (n=90), BEAM +/- ATG (n= 75), Cy plus radiation +/- ATG (n=40) and Busulphan plus Cy +/- ATG. Ten patients received fludarabine plus other agents.

An actuarially adjusted one year transplant (procedure) related mortality (TRM) of 7% (4-10) was observed, with a trend toward more toxicity with the more severe regimens. A parallel increased efficacy regarding either remission induction or maintenance has not yet emerged. Complications and causes of death have been as expected from previous HSCT experience (mostly infection, bleeding and organ toxicity), apart from a suggestion that the heart in SSc is especially sensitive to chemotherapeutic agents.

Outcome was different for the different ADs, and generalisations are not possible.

In SSc 70% of patients achieved a > 25% improvement of the initial skin score (in most cases durable) , with a tendency to stabilisation of lung function (2). The TRM of the first 45 cases was 17% , which fell to 12.5% in a second analysis of 65 cases and is predicted at 7.5% using the new selection criteria. Patients with a mean pulmonary artery pressure (PAP) of > 50 mm Hg did poorly, both in terms of transplant related adverse events and lack of reponse. However, in some patients the mean PAP fell after HSCT with symptomatic improvement. Based on the phase I/II data a randomised prospective controlled trial has commenced: Autologous Stem cell Transplantation International Scleroderma (ASTIS) Trial, comparing HSCT (Cy 4g/m^2 mobilisation, Cy 200mg/kg and rabbit ATG 7.5mg/kg conditioning with a CD34 selected graft versus monthly pulse Cy 750mg/m^2 for 12 months. (See website: www.astistrial.com). The primary end point is event free 2 year survival.

An analysis of the first 85 MS patients showed a progression free survival of 74% (+/- 12) at three years overall, being higher in secondary progressive (78%) than primary progressive (66%) MS. In 78 patients data were available concerning MRI gadolinium enhancing or expanding lesions. In those patients with active lesions preHSCT, these resolved. Three patients experienced transient disease flare during mobilisation with G-CSF, and 22 following HSCT. These were mostly transient in all but 6. There were 7 deaths, 5 TRM and 2 progressive disease. A randomised controlled trial is being planned: Autologous Stem cell Transplantation International Multiple Sclerosis (ASTIMS) Trial, comparing BEAM and ATG and an unmanipulated graft against mitoxanthrone.

In RA and SLE most patients responded to HSCT but relapse was seen in around two thirds. In the first 76 RA patients, all of whom had failed multiple disease modifying antirheumatic drugs (DMARDs), 67% achieved a 50% improvement in a panel of response parameters (ACR50), and in those relapsing, a response to DMARDs which were ineffective pre-transplant, was often observed. Most received Cy 200mg/kg as conditioning with an unmanipulated graft in 28. There was only one TRM, a patient with Busulphan / Cy and an incidental lung carcinoma. A randomised controlled trial: Autologous Stem cell International Rheumatoid Arthritis (ASTIRA) Trial will soon begin in which all patients are mobilised with Cy 4g/m^2 and then either HSCT (Cy 200mg /kg and an unmanipulated graft) or continued DMARD treatment.

A preliminary review of the SLE data (48 evaluable patients) showed that all respond, with a relapse in around 40%. As with RA, many relapses responded to simple agents, and average daily corticosteroid dose could be reduced in many. A TRM of 11% in this first series was seen, probably reflecting the fact that such patients are seriously ill with vital organ involvement at the time of transplant. More phase II data concerning patient selection and maintenance therapy post transplantation will be undertaken.

JIA patients have shown a sensitivity to toxicity (macrophage activation syndrome) if systemically active at the time of transplant. Many responded well (15 complete and 3 partial remissions) and further phase II data is being generated concerning non radiation-based regimens and patient selection.

Data on 16 cases of refractory autoimmune cytopenias showed a response in 8, four of whom experienced complete remission. There were 3 deaths, 2 from haemorrhage and 1 haemolysis.

The EBMT collects data on all non USA autoimmune disease patients undergoing HSCT.

Session 3: Genetics of experimental and human SLE and RA
Chair: Martin Weigert

From mouse to man: understanding human inflammatory disease

Gary Peltz

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The integrated use of a novel SNP-based genotyping method and gene expression analysis using high-density oligonucleotide microarrays has markedly increased the rate at which complex biological processes can be analyzed. Application of these technologies to murine experimental models of human inflammatory disease has enabled genetic susceptibility loci to be rapidly identified. The identified murine susceptibility genes provide insight into pathways regulating human inflammatory disease susceptibility. An example of a genetic locus for asthma susceptibility identified in mouse, and the genetic analysis of human asthmatic cohorts will be presented. Complex trait analysis in mice was also accelerated by our computational, haplotype-based method for predicting chromosomal regions regulating murine complex traits.

Role of PD-1 in immune tolerance and tumor surveillance

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PD-1 is a receptor of the immunoglobulin superfamily that negatively regulates T-cell antigen receptor (TCR)-signaling by interacting with the specific ligands (PD-L), and is suggested to play a role in the maintenance of self-tolerance. We have recently reported that PD-1 immuno-inhibitory co-receptor deficient mice develop autoimmune dilated cardiomyopathy in the BALB/c background with high-titer autoantibodies against a heart specific 30kda protein. In this study, we purified the 30kda protein from heart extract and identified it as cardiac troponin I (ctni). Administration of monoclonal antibodies against ctni induced dilatation and dysfunction of heart in wild type mice. Addition of monoclonal antibodies against ctni augmented the voltage dependent Ca^{2+} current of normal cardiomyocytes. These findings suggest that antibodies against ctni induce dysfunction and dilatation of heart by increasing Ca^{2+} current of cardiomyocytes.

We also examined possible roles of the PD-1/PD-L system in tumor immunity. Transgenic expression of PD-L1, one of the PD-L, in P815 tumor cells rendered them less susceptible to the specific TCR-mediated lysis by cytotoxic T cells in vitro, and markedly enhanced their tumorigenesis and invasiveness in vivo in the syngeneic hosts as compared with the parental tumor cells that lacked endogenous PD-L. Both effects could be reversed by anti-PD-L1 antibody. Survey of murine tumor lines revealed that all the myeloma cell lines examined naturally expressed PD-L1. Growth of the myeloma cells in normal syngeneic mice was inhibited significantly albeit transiently by the administration of anti-PD-L1 antibody in vivo, and was suppressed completely in the syngeneic PD-1-deficient mice. These results suggest that the expression of PD-L1 can serve as a potent mechanism for potentially immunogenic tumors to escape from host immune responses, and that blockade of interaction between PD-1 and PD-L may provide a promising strategy for specific tumor immunotherapy.

Apoptosis in rheumatic diseases

Keith B Elkon

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Genetic studies in mice indicate that predisposition to lupus-like diseases is caused by at least three mechanisms: 1) alterations in the threshold of activation of lymphocytes or macrophages 2) defective signaling for activation-induced cell death and 3) reduced clearance of apoptotic cells. To define the mechanisms whereby mice with deficiencies in either C1q, SAP (the mouse counterpart of CRP) or serum IgM develop lupus, we studied the efficiency of phagocytosis of apoptotic cells *in vitro* and *in vivo* in mice with varying levels of C1q, CRP or IgM and also examined the immune response to ingestion of dying cells under these conditions.

Deficiency of C1q led to impaired macrophage phagocytosis of apoptotic cells whereas CRP augmented phagocytosis, largely through recruitment of the early complement components. Like CRP, normal polyclonal IgM bound to apoptotic cells and activated complement on the cell surface. Direct binding as well as absorption experiments revealed that CRP and IgM antibodies had a similar ligand recognition specificity, namely lysophospholipids containing phosphorylcholine. These findings suggest that phospholipid exposure on apoptotic cells promote opsonization by serum proteins, including natural antibodies, leading to activation of complement, macrophage ingestion and T cell suppression. We discuss how genetic deficiencies of opsonins or processing of dying cells leads to autoimmunity.

Rheumatoid arthritis is associated with a tumor like growth of the synovial pannus. Failure to downregulate the growth of the pannus may be explained not only by a relative paucity of regulatory death inducers such as Fas ligand, but also by a partial resistance to Fas and other death pathways. Growth and survival of fibroblast like synovial cells is due to exposure to pro-inflammatory cytokines such as TNF-∀ and IL-1∃ that predominantly drive the NFkB pathway. TGFβ also drives fibroblast growth and we have recently shown that TGFβ may exert its role by activating a pivotal growth promoting kinase, Akt (protein kinase B). Genetic variation in cytokine production or response, have been proposed to account for exaggerated response to inflammatory stimuli in patients with RA.

Function and dynamic of regulatory T cells during inflammatory responses

J. Demengeot, I. Caramalho, T. Carvalho, M. Baretto, S. Zelenay, F. Fontes

Immune regulatory cells encompassed in the $CD4^+CD25^+$ subset of T lymphocytes control both autoimmune reactions and protective immune responses. Accordingly, we have shown that these cells prevent lethal inflammatory pneumonia in *Pneumocystis Carinii* infected mice. Regulatory T cells not only reduce the expansion of naïve CD4+ T cells and the local inflammation induced by infectious agents, but also the effective elimination of the pathogen. We further demonstrated that regulatory T cells modulate inflammatory responses in the absence of non-self antigens.

Strikingly, inflammation triggers regulatory T cell expansion and effector functions and this trigger is mediated not only by inflammatory cytokines but also directly by ligands of Toll like receptors.

These results, by providing a novel molecular basis for regulatory T cell dynamic, are now explored to design therapeutic strategies based on the inhibition or enhancement of Treg activities for the management of either chronic infection and tumor immunity or autoimmune disease. Our analyses of a cohort of human SLE patients revealed a significant quantitative deficiency in their $CD4^+CD25^+$ subset, confirming that these approaches should be highly relevant for human SLE treatment.

Session 4: Potential targets for therapeutic intervention
Chair: Juan J. Lafaille

Regulation of the immune response at the cellular and molecular level

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The immune response has evolved to protect us from infectious agents. All multi-cellular organisms have some form of innate immunity but higher organisms have developed an adaptive immune response consisting of cellular and humoral factors, which serve to provide a memory response, which is protective against a variety of subsequent infections. Because random processes are used to generate the adaptive immune response, it is not possible to prevent the production of lymphocytes that are self-reactive. Because of this, numerous protective mechanisms have evolved. These include the elimination of some auto reactive lymphocytes by deletion in the thymus, peripheral tolerance mechanisms which lead to the elimination of autoreactive cells in the periphery or their inactivation and finally dominant inhibitory mechanisms. Recent studies of dominant inhibitory mechanisms have shown that both the cytokines IL10 and TGF beta, as well as a variety of cell populations including CD4+ CD25+ regulatory T cells, Nk T cells, gamma delta T cells and so on mediate protection against self-reactivity. In this lecture I will discuss immunoregulatory mechanisms and the importance that they play in the immune response to infectious agents, self-antigens and the specific case of tumor antigens. Further, I will discuss the regulation of the innate immune response and the role that plays in immunoregulation in general.

CD69 down-regulates immune reactivity through active TGF-beta production in collagen-induced arthritis

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CD69 is induced following activation of leukocytes at inflammatory sites, but its physiological role remains unknown. We explored the role of CD69 in the immune reactivity by analyzing a model of collagen-induced arthritis (CIA) in wild type and CD69-deficient mice. $CD69^{-/-}$ mice showed higher incidence and severity of CIA, with exacerbated immune response to type II collagen. TGF-beta1, which is protective in CIA, was reduced in $CD69^{-/-}$ mice inflammatory foci. Local injection of blocking anti-TGF-beta increased CIA severity in $CD69^{+/+}$, but not in $CD69^{-/-}$ mice. Moreover, *in vitro* engagement of CD69 induced TGF-beta1 production both in mouse and human synovial leukocytes. Our results unveil CD69 as a negative modulator of immune reactivity and inflammation through TGF-beta synthesis that regulates other pro-inflammatory cytokines, such as IL-1beta or RANTES.

The role of CD69 as a negative modulator of immune reactivity opens the possibility of an interesting and novel approach for the therapy of chronic inflammation and other immune-mediated diseases by targeting CD69.

In addition, since CD69 is selectively expressed in activated leukocytes infiltrating inflamed tissues, the pharmacological stimulation of TGF-beta synthesis through CD69 may have a localized effect, thus avoiding the detrimental consequences of a systemic TGF-beta up-regulation. As for other regulatory molecules (e.g., CTLA-4), CD69 could be an interesting target for the therapy of immune-mediated diseases.

Experimental immune-interventions in inflammatory diseases with heat shock proteins and their derivative peptides

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Antigen specific immunotherapies of autoimmune diseases have been developed and tested, for a major part, along the principles of oral tolerance. However, partly due to difficulties in the proper definition of relevant autoantigens and the monitoring of immune effects at the level of antigen specific T cells, results have been unsatisfactory sofar [1]. With the advent of novel technologies for the tracking of antigen specific T cells, also in humans, there are renewed chances for clinical research oriented towards the development of antigen specific immune interventions [2]. In addition, the current wide interest in the definition and characterisation of antigen specific regulatory T cells as key-players in the control of peripheral tolerance, is in itself a trigger to renewed interest in the use of self (related) antigens for the therapeutic (or preventive!) control of autoimmune diseases.

Heat shock proteins, also called stress-proteins, are ubiquitous self-antigens that become over-expressed in inflamed tissues. For some reason, the prokaryotic homologous proteins, present in every bacterial species, are dominantly immunogenic. This is striking, especially given the fact that these proteins have large areas of sequence homologies with the host (mammalian) counterparts. Furthermore, in various experimental models of autoimmune diseases, immunisation with bacterial heat shock proteins has been seen to lead to inhibition of disease development [3-5]. In addition oral or nasal administration has similarly been seen to lead to disease inhibition [6-9]. Based on the experimental evidence collected sofar, it becomes attractive to suppose that the exposure to homologues of these self antigens, as present in for instance the bacterial intestinal flora, has a decisive impact on the regulation of self tolerance at the level of T cells [10]. If so, it becomes attractive to use such proteins or their derivative peptides [11] for modulation of inflammation relevant T cells as an antigen specific immunotherapy approach, without the immediate necessity of defining disease specific auto-antigens.

References:

1. W. van Eden, M. de Boer, J.W.J. Bijlsma, R.R.P de Vries, C.J.M. Melief (Eds.) Specific Immunotherapy of Chronic Autoimmune diseases. How to translate the Experience of experimental models into novel treatment modalities. KNAW Verhandelingen, Afd. Natuurkunde, Tweede Reeks, deel 101. Amsterdam (1999).

2. Prakken B, Wauben M, Genini D, Samodal R, Barnett J, Mendivil A, Leoni L, Albani S. Artificial antigen-presenting cells as a tool to exploit the immune 'synapse'. Nat Med. 2000; 6:1406-10.

3. Anderton SM, R. van der Zee, B. Prakken, A. Noordzij, W. van Eden. Activation of T cells recognizing self 60kDa heat shock protein can protect against experimental arthritis. J. Exp. Med. 1995; 181: 943-952.

4. Prakken BJ, U. Wendling, R. van der Zee, V.P. Rutten, W. Kuis, W. van Eden. Induction of IL-10 and inhibition of experimental arthritis are specific features of microbial heat shock proteins that are absent for other evolutionarily conserved immunodominant proteins. J Immunol. 2001; 167:4147-4153.

5. Corrigall VM, M.D. Bodman-Smith, M.S. Fife, B. Canas, L.K. Myers, P. Wooley, C. Soh, N.A. Staines, D.J. Pappin, S.E. Berlo, W. van Eden, R. van Der Zee, J.S. Lanchbury, G.S. Panayi. The human endoplasmic reticulum molecular chaperone BiP is an autoantigen for rheumatoid arthritis ritis and prevents the induction of experimental arthritis. J Immunol. 2001; 166:1492-1498.

6. Prakken ABJ, R. van der Zee, S.M. Anderton, P.J.S. van Kooten, W. Kuis, W. van Eden. Peptide induced nasal tolerance for a mycobacterial hsp60 T cell epitope in rats suppresses both adjuvant arthritis and non-microbially induced experimental arthritis. PNAS USA 1997; 94: 3284-3289.

7. Cobelens PM, C.J. Heijnen, E.E.S. Nieuwenhuis, P.P.G. Kramer, R. van der Zee, R, W. van Eden, A. Kavelaars. Treatment of adjuvant-induced arthritis by oral administration of mycobacterial hsp65 during disease. Arthritis & Rheum 2000; 43: 2694-2702.

8. Wendling U, L. Paul, R. van der Zee, B. Prakken, M. Singh, W. van Eden. A conserved mycobacterial heat shock protein (hsp) 70 sequence prevents adjuvant arthritis upon nasal administration and induces IL10-producing T cells that cross-react with the mammalian self-hsp70 homologue. J. Immunol. 2000; 164: 2711-2717.

9. Prakken BJ, Roord S, van Kooten PJ, Wagenaar JP, van Eden W, Albani S, Wauben MH. Inhibition of adjuvant-induced arthritis by interleukin-10-driven regulatory cells induced via nasal administration of a peptide analogue of an arthritis-related heat-shock protein 60 T cell epitope. Arthritis Rheum. 2002;46:1937-46.

10. Paul AGA, P.J.S. van Kooten, W. van Eden, R. van der Zee. Highly autoproliferative T cells specific for 60-kDa heat shock protein produce IL-4/IL-10 and IFN-α and are protective in adjuvant arthritis. J Immunol 2000; 165: 7270-7277.

11. Kamphuis SS, W. de Jager, I.M. de Kleer, G. Gordon, E. Koffeman, M. Massa, A. Martini, G.T. Rijkers, W. van Eden, W. Kuis, A.B. Prakken, S. Albani. V1.2. recognition of multiple hsp60 epitopes in patients with juvenile idiopathic arthritis opens the way for antigen-specific immunotherapy. Ann Rheum Dis 2001; 60 Suppl 1:II7-II15.

Control of regulatory T cell development by the transcription factor FOXP3

Shohei Hori, Takashi Nomura, and Shimon Sakaguchi

Regulatory T cells are engaged in the maintenance of immunological self-tolerance and the prevention of deleterious inflammatory responses against foreign antigens. Little is known, however, about the molecular mechanism of their development. Here we show that Foxp3, which encodes a transcription factor genetically defective in an autoimmune/inflammatory syndrome in humans and mice, is specifically expressed in naturally occuring $CD25^{+}CD4^{+}$ regulatory T cells. Furthermore, retroviral gene transfer of Foxp3 converts naïve T cells towards a regulatory T cell phenotype similar to naturally occurring $CD4^{+}$ regulatory T cells. Thus, Foxp3 is a master regulatory gene for the development of regulatory T cells.

Session 5: Novel therapies
Chair: António Coutinho

Lymphocytes as therapeutic targets in autoimmune disease

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'Biological' therapeutics (monoclonal antibodies [mAbs] and receptor-fusion proteins) provide powerful tools for therapy of autoimmune disease. My presentation will cover several aspects of biological therapy:

1. Manipulating cells. In the context of autoimmunity, the central pathogenic cells are the lymphocytes. Historically, depleting mAbs were used to target these cells. The theory was that elimination of pathogenic clones would be followed by reconstitution of a 'new' immune system that lacked autoreactive potential. A mAb that recognises CD52 (CAMPATH-1H) was widely used for autoimmunity, with variable results depending on the condition under treatment. The effects were transient in rheumatoid arthritis (RA) but longer remissions were documented in vasculitis, inflammatory eye disease and multiple sclerosis. Currently anti-CD20 (Rituximab) is being studied in RA and systemic lupus erythematosus. This mAb specifically eliminates B-cells. LFA3-Ig is a depleting mAb-like molecule that targets CD2, a molecule that is up-regulated on memory T-cells. This has shown promise in psoriasis and psoriatic arthritis.

Depleting therapies that targetted T-cells lost popularity when it was recognised that reconstitution was often incomplete, potentially resulting in immunodeficiency. It was also discovered that non-depleting anti-T cell mAbs could have tolerogenic effects, and so the focus shifted in favour of such reagents. A number of surface molecules on T-cells have been targetted including CD4, CD3, and CD28. In animals, such mAbs have extremely powerful immunomodulatory effects, inducing tolerance to allografts, switching off autoimmune disease, and inducing regulatory T-cells. Effects have been less impressive in man but recent trials in insulin-dependent diabetes are starting to suggest tolerogenic effects. Thus, a 2 week course of a non-depleting anti-CD3 mAb in recent-onset diabetics resulted in sustained pancreatic insulin production for 12 months compared to a control group.

2. Therapeutic tolerance induction. It is important to realise that we are still learning how best to use these biological therapies and the rules governing treatment are very different to those for a conventional small molecule drug. For example, we do not know the most appropriate regimes or protocols for tolerance induction in man. The diabetes trials suggest that we are moving closer to our target of switching off autoimmunity but we badly need surrogate markers with which to guide therapy. Thus, whereas a fall in CRP or ESR suggests that an anti-inflammatory treatment is working, we have no equivalent tests for a tolerogenic therapy. Under these circumstances we assume that the treatment has not worked if the disease does

not remit. Some patients, however, seem to respond more favourably to conventional treatments after immunotherapy than they did before it – even when the therapy itself seemed ineffective at the time of administration. This suggests that the treatment may have had an immunomodulatory effect and we now need to discover surrogate laboratory markers that correlate with such clinical observations. These can then be used to guide our choice of therapy and regime.

3. Unwanted biological activities. The side effects of mAbs can be acute, such as first-dose reactions or more chronic, such as delayed lymphocyte reconstitution and immunogenicity. As we begin to understand the causes of, and mechanisms underlying, these reactions it becomes possible to rationally design novel mAbs to counteract or avoid them. Many mAbs in clinical use now have mutated 'constant' regions to prevent interaction with, for example, Fc-gamma receptors. Aglycosyl mAbs, for example, lack carbohydrate side chains which are important for interaction with both complement and Fc-gamma receptors. We are also beginning to witness pharmacogenetic phenomena linked to immune system polymorphisms. For example, Rituximab binds CD16, the intermediate affinity Fc-gamma receptor. This interaction is important in the elimination of target cells. In patients with B-cell lymphoma treated with this mAb, those with a higher affinity allotype of CD16 demonstrated an improved therapeutic response. We have seen a similar phenomena relating to T-cell depletion by anti-CD4 and CAMPATH-1H mAbs.

NALPs, a protein family involved in the activation of proinflammatory caspases, are mutated in auto-inflammatory diseases

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Generation of Interleukin (IL)-1β via cleavage of its pro-form requires the activity of caspase-1 (and caspase-11 in mice), but the mechanism involved in the activation of the pro-inflammatory caspases remains elusive. A newly discovered family of cytoplasmic proteins — the NALPs — has been implicated in the activation of caspase-1 by the Toll-like receptors (TLRs) during the response to microbial infection. Like the structurally related Apaf-1, which is responsible for the activation of caspase-9, the NALP1 protein forms a large, signal-induced multiprotein complex, the inflammasome, resulting in the activation of pro-inflammatory caspases. The inflammasome comprises caspase-1, caspase-5, Asc and NALP1. Expression of a dominant-negative form of Asc in THP-1 cells blocks proIL-1β maturation and activation of inflammatory caspases induced by LPS *in vivo*. Thus the inflammasome constitutes an important arm of the innate immunity.

There is *in vivo* evidence for a crucial role of NALP family members in inflammation. Patients with hereditary fever syndromes and chronic inflammatory diseases carrying mutations in NALP3 have recently been identified.

References:

Aganna, E., Martinon, F., Hawkins, P. N., Ross, J. B., Swan, D. C., Booth, D. R., Lachmann, H. J., Bybee, A., Gaudet, R., Woo, P., et al. (2002). Association of mutations in the NALP3/CIAS1/PYPAF1 gene with a broad phenotype including recurrent fever, cold sensitivity, sensorineural deafness, and AA amyloidosis. Arthritis Rheum. *46*, 2445-2452.

Martinon, F., Burns, K., and Tschopp, J. (2002). The Inflammasome. A Molecular Platform Triggering Activation of Inflammatory Caspases and Processing of proIL-beta. Mol. Cell *10*, 417.

Tschopp, J., Martinon, F., and Burns, K. (2003). NALPs: a novel protein family involved in inflammation. Nat Rev Mol Cell Biol *4*, 95-104.

POSTERS

Paradoxical effects of arthritis regulating chromosome 4 regions on myelin oligodendrocyte glycoprotein induced encephalomyelitis in congenic rats

Becanovic K, Bäckdahl L, Wallström E, Aboul-Enein F, Lassmann H, Olsson T, Lorentzen J C

Definition of genes regulating experimental organ-specific inflammatory diseases may lead to development of therapy for human diseases such as rheumatoid arthritis and multiple sclerosis (MS). We here studied the genetic regulation of myelin oligodendrocyte glycoprotein (MOG)-induced experimental autoimmune encephalomyelitis (EAE) in rat strains congenic for arthritis-regulating genome regions on chromosome 4(1). We used a congenic rat strain with a 70 cM fragment from the EAE- and arthritis-resistant PVG.1AV1 rat strain on the arthritis- and EAE-permissive DA rat background. In addition, we used three intra-regional recombinant strains, C4R1-C4R3, which overlap with arthritis linked loci.

C4R1 and C4R2 overlap with Cia3(2) and Pia5(3), and C4R1 overlap with Oia2(4). Interestingly, PVG.1AV1 alleles in the C4R1 recombinant, did not affect arthritis in this strain combination, but conferred protection against MOG-EAE. In contrast, PVG.1AV1 alleles in C4R2 mediate down-regulation of arthritis in males, but the region had no effect in MOG-EAE. Paradoxically, PVG.1AV1 alleles in the C4R3 recombinant down-regulated arthritis, but the same region aggravated MOG-EAE and conferred a more intense humoral autoimmune response against MOG. Since we did not observe regulation in the full-length 70 cM congene the different directions of disease regulation in C4R1 and C4R3 appear to counteract each other. We thus provide original evidence that genome regions can have opposite effects in different organ-specific inflammatory diseases. This co-localisation of loci exerting disease-regulating effects could be due to a cluster of different regulating genes, or due to a single gene. These observations may be of general interest for scientists, in the field of genetic dissection of disease regulation using congenic strains.

References:

1. Bäckdahl L, Ribbhammar U, Lorentzen J C. Mapping and functional char Mapping and Functional Characterization of Rat Chromosome 4 Regions that Regulate Arthritis Models and Phenotypes in Congenic Strains. Arthritis Rheum 2003 in press.
2. Remmers EF, Longman RE, Du Y, O'Hare A, Cannon GW, Griffiths MM, et al. A genome scan localizes five non-MHC loci controlling collagen-induced arthritis in rats. Nat Genet 1996;14:82-5.
3. Vingsbo-Lundberg C, Nordquist N, Olofsson P, Sundvall M, Saxne T, Pettersson U, et al. Genetic control of arthritis onset, severity and chronicity in a model for rheumatoid arthritis in rats. Nat Genet 1998;20:401-4.
4. Lorentzen JC, Glaser A, Jacobsson L, Galli J, Fakhrai-rad H, Klareskog L, et al. Identification of rat susceptibility loci for adjuvant-oil-induced arthritis. Proc Natl Acad Sci U S A 1998;95:6383-7.

Evidence for CTLA4 as a susceptibility gene for SLE

M Barreto, C Fesel, R Ferreira, F Fontes, C Pereira, B Martins, R Andreia, JF Viana, F Crespo, C Vasconcelos, AM Vicente and C Ferreira

Systemic Lupus Erythematosus (SLE) is a genetically complex autoimmune disorder of unknown etiology. It is widely accepted that dysregulation of the co-stimulatory system contributes to the initiation and maintenance of autoimmunity due to the activation of self-reactive T cells. The CTLA4 molecule, which has a role in down-regulating the activation of T cells, has been implicated in a number of autoimmune diseases such as Type 1 Diabetes and Multiple Sclerosis. With the purpose of investigating the role of CTLA4 in the pathogenesis of SLE we genotyped two polymorphic markers within the CTLA4 gene (a SNP involving an A to G transition in exon 1 and a microsatelite in the 3'UTR), in a sample of 91 patients and 135 controls. These patients originate from Portugal and include 95% females with an age range of 16-60y (mean age 39y). We found a significant association for the microsatelite marker with SLE (χ^2=36.241, $p<0.001$), with a specific allele showing a protective effect (χ^2=16.261, $p<0.001$) and the group of rare alleles significantly contributing to the susceptibility to the disease (χ^2=12.081, $p<0.001$). When the patient and control populations were matched for sex because of the high prevalence of female patients, the significant association was still confirmed (χ^2=26.678, $p<0.001$). No association was found with the exon 1 SNP. Because the 3'UTR polymorphism may be involved in the regulation of CTLA4 expression, we hypothesize that the genotype-dependent defective expression of this molecule may be responsible for a deficient down regulation of the immune response, leading to autoimmunity in SLE.

Regulatory T cells selectively express toll like receptors and are activated by lipopolysaccharide

I. Caramalho, T. Lopes-Carvalho, D. Ostler, S. Zelenay, M. Haury and J. Demengeot

Regulatory CD4 T cells (Treg) have been shown to play a crucial role in the prevention of autoimmunity (1, 2) and in the control inflammatory reactions to both commensal bacteria (3) and opportunist pathogens (4).

Activation of Treg functions during these processes might be mediated by host-derived pro-inflammatory molecules, or directly by bacterial products.

We tested the hypothesis that engagement of germline encoded receptors expressed by Treg participate in the triggering of their function. We report that the subset of CD4 cells known to exert regulatory functions *in vivo* (CD45RBlow, $CD25^+$) (5, 6) selectively express Toll Like Receptor (TLR)-4, -5, -7 and -8. Exposure of $CD4^+CD25^+$ cells to the TLR-4 ligand lipopolysaccharide (LPS) induces up-regulation of several activation markers and enhances their survival/proliferation. This proliferative response does not require APC and is augmented by TCR triggering and IL-2 stimulation. Most importantly, LPS treatment increases $CD4^+CD25^+$ cells suppressor efficiency by 10 folds, and reveals suppressive activity in the $CD4^+$CD45RBlow $CD25^-$ subset that, when tested *ex-vivo*, score negative in this assay. Moreover, LPS-activated Tregs efficiently control naïve CD4 T cell-dependent wasting disease.

These findings provide the first evidence that Treg respond directly to pro-inflammatory bacterial products, a mechanism that likely contributes to the control of inflammatory responses.

References:

1) Takahashi T, Y Kuniyasu, M Toda, N Sakaguchi, M Itoh, M Iwata, J Shimizu and S Sakaguchi. "Immunologic self-tolerance maintained by $CD25^+CD4^+$ naturally anergic and suppressive T cells: induction of autoimmune disease by breaking their anergic/suppressive state". Int Immunol, 1998, 10:1969-80.

2) Sakaguchi S, N Sakaguchi, J Shimizu, S Yamazaki, T Sakihama, M Itoh, Y Kuniyasu, T Nomura, M Toda and T Takahashi. "Immunologic tolerance maintained by $CD4^+CD25^+$ regulatory T cells: their common role in controlling autoimmunity, tumor immunity and transplantation tolerance". Immunol Rev, 2001, 182: 18-32.

3) Powrie F, J Carlino, MW Leach, S Mauze and RL Coffman. "A critical role for transforming growth factor-beta but not interleukin 4 in the suppression of T helper type 1-mediated colitis by CD45RB(low) $CD4^+$ T cells". J Exp Med, 1996, 183:2669-74.

4) Hori S, TL Carvalho and J Demengeot. "CD25+CD4+ regulatory T cells suppress $CD4^+$ T cell-mediated pulmonary hyperinflammation driven by *Pneumocystis carinii* in immunodeficient mice". Eur J Immunol, 2002, 32:1282-9.

5) Sakaguchi S, N Sakaguchi, M Asano, M Itoh and M Toda. "Immunologic self-tolerance maintained by activated T cells expressing IL-2 receptor alpha-chains (CD25). Breakdown of a single mechanism of self-tolerance causes various autoimmune diseases". J Immunol, 1995, 155:1151-64.

6) Read S, V Malmstrom and F Powrie. "Cytotoxic T lymphocyte-associated antigen 4 plays an essential role in the function of $CD25(^+)CD4(^+)$ regulatory cells that control intestinal inflammation". J Exp Med, 2000,192:295-302.

Multivariate analysis and genetics of autoantibody repertoires in Systemic Lupus Erythematosus (SLE) multiplex families

M Barreto, C Fesel, R Ferreira, F Fontes, R Andreia, JF Viana, F Crespo, C Vasconcelos, C Ferreira and AM Vicente

SLE is an autoimmune disease with strong female predominance and demonstrated inheritable effects. However, this disease is complex, with multiple genes and environmental factors contributing to its likely heterogeneous etiology. The production of IgG autoantibodies to a wide variety of primarily nuclear and non-nuclear autoantigens, likely to include yet uncharacterized ones, is a main characteristic of patients, and is also found in unaffected relatives. In order to identify genetic factors involved in the pathophysiology of SLE, we have applied an indirect strategy that characterizes serological subtypes in patients and relatives, and then analyzes the corresponding inheritance patterns, thus aiming at the definition of genetically simpler endophenotypes suitable for genetic mapping. For this purpose, in a sample of 27 multiplex families that includes 46 SLE patients and 134 unaffected relatives, and in 52 unrelated patients and 60 healthy controls, we measured serum IgG and IgM autoantibody repertoires to several tissue extracts and cellular fractions, employing a standardized quantitative immunoblot method. This technique allows the quantification of a large number of separate reactivity bands, and was complemented with antigen-specific quantitative ELISAs for known SLE-related reactivities. Principal component analysis (PCA) on these parameters and corresponding clinical data showed that genetic and non-genetic effects are largely represented by distinct principal components. It is striking that, generally, non-genetic PCA factors represent more information and are more informative for discrimination of SLE patients. For multiple subsequent principal components there was evidence for heritability. These results, though still preliminary and being extended in a larger sample, support the existence of SLE-related traits, which are defined by multiple parameters, show an inheritance pattern determined by one or very few genes and can be characterized by multiparametric autoantibody reactivity patterns. This strategy is therefore proving useful for the identification of SLE-related phenotypic traits suitable for genetic mapping.

The negative effect of steroids and cyclophosphamide on regulatory T cells

MF. Fontes and J. Demengeot

Regulatory T cells (Treg) protect from autoimmune disease (AID) in various experimental models and are reduced in human autoimmune diabetes.

Immunesuppressants (IS) such as steroids and cyclophosphamide (CYP) are widely used to treat AID but potentially interfere with Treg homeostasis and function. Among other effects, steroids alter the genetic control of cytokine synthesis and alkylating agents preferentially affect cycling cells.

Anti-myelin basic protein (MBP) TCR transgenic mice (T/R^+) remain healthy unless deficient for the RAG-1 gene (T/R^-), when autoimmune encephalomyelitis (EAE) spontaneously develops. T/R^- mice are protected from EAE by adoptive transfer of a minimum of 3 X 105 $CD4^+$ cells from wild type donors, enriched for Treg. Among other mechanisms, Treg suppress effector cell function through inhibition of IL-2 and are enriched for proliferating cells *in vivo*.

We have used this experimental system to study the effect of hydrocortisone (HC) and CYP on regulatory function. We show that T/R^- mice are not protected from EAE when transferred with 3 X 105 $CD4^+$ cells purified from HC and CYP (single or combination) treated donors. FACS analysis of T/R^+ mice treated with the same protocol of HC and CYP reveals that combination therapy followed by CYP alone causes the greatest reduction in CD25+/CD4+ T cells. Induction of EAE in T/R+, with HC and CYP and with CYP alone occurred in a dose and age related manner.

The MBP transgenic mouse is an experimental model for human multiple sclerosis. The deleterious effect of steroids and CYP on Treg may explain why, even though steroids and CYP are effective therapy for relapses in MS, they do not seem to affect the progression of the illness. These findings may influence the treatment of human autoimmune disease.

The autoantigen HB, a protein that must be bound to DNA to be recognized by autoantibodies, is the Barrier to Autointegration Factor

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In our laboratory we are presently using new approaches to identify autoantigens by means of proteomic tools. In this paper we describe the identification of a new autoantigen. This autoantigen, named HB, was found in a patient with rheumatoid arthritis, by means of a specific protocol developed in our laboratory for the study of DNA-binding proteins. The HB antigen was identified as a complex of three proteins of 9,000, 7,500 and 7,000 Da. The 9,000 and 7,500-Da proteins were phosphorylated.

Interestingly, the autoantibodies recognize only the 7,000 Da component of the complex, and this protein must be bound to double stranded DNA to be recognized by the autoantibodies. Therefore, two conclusions can be reached. Firstly, the antigen undergoes a conformational change after its binding to DNA and, secondly, the autoantibody response is restricted to this DNA-protein complex. The 7,000 Da antigen was identified by 2-D electrophoresis and mass spectrometry as the protein BAF (barrier-to-autointegration factor), a protein implicated in the inhibition of autointegration of retrovirus, such as HIV, and in the cellular cycle. The physicochemical characteristics described for BAF, its ability to bind dsDNA but not ssDNA, and its perinuclear localization confirm that HB is BAF.

Enhanced antitumor immunity in mice deficient in CD69

Enric Esplugues, David Sancho, Javier Vega-Ramos, Carlos Martínez-A., Uta Syrbe, Alf Hamann, Pablo Engel, Francisco Sánchez-Madrid, Pilar Lauzurica

We investigated the *in vivo* role of CD69 by analyzing the susceptibility of $CD69^{-/-}$ mice to tumors. $CD69^{-/-}$ mice challenged with MHC class I-tumors (RMA-S, RM-1) showed greatly reduced tumor growth and prolonged survival, compared to wt mice. The enhanced anti-tumor response was NK cell- and T lymphocyte- mediated and persisted in immuno-compromised $CD69^{-/-}$ RAG-negative mice. Resistance of $CD69^{-/-}$ mice to MHC class I- tumor growth was also associated with increased production of the chemokine MCP-1, diminished TGF- production and decreased lymphocyte apoptosis. Moreover, the *in vivo* blockade of TGF- in wt mice resulted in enhanced anti-tumor response, directly implicating the diminished production of TGF- found in $CD69^{-/-}$ mice as a basis of their enhanced anti-tumor immunity. In addition, TGF is regulated by CD69 signaling in NK and T lymphocytes. CD69 engagement induced total and active TGF-1 production in different lymphocytes subsets establishing a direct link between CD69 engagement and TGF- synthesis, and explaining the higher TGF-1 levels found in $CD69^{+/+}$ mice compared to $CD69^{-/-}$ mice. These data demonstrate an inhibitory role for CD69 in immune reactivity through the regulation of TGF- synthesis and open the possibility of an interesting and novel approach for the therapy of cancer, chronic inflammation and other immune-mediated diseases by targeting CD69.

Small-molecular compounds enhance the loading of APC with the encephalitogenic MBP protein

Viviana Marin Esteban, Kirsten Falk and Olaf Roetzschke

Small-molecular compounds with hydrogen bond (H-bond) donor function are able to trigger exchange reactions of MHC class-II ligands. Here, we show that their effect is not limited to short peptides. Also encephalitogenic myelin basic protein (MBP) is transferred with great efficiency onto HLA-DR molecules when H-bond donor molecules such as parachlorphenol (pCP) are present. The effect was observed not only with soluble MHC class II but also with HLA-DR1 and HLA-DR2 molecules on the cell surface. The improved loading of APC translates directly into improved T cell activation. In the presence of pCP T cells reacted at significantly lower antigen concentrations, an effect observed with purified MBP protein as well as with crude spinal cord homogenate. The 'accidental' transfer of autoantigens such as MBP onto activated APC might trigger fatal autoimmune reactions and small molecules as catalysts of this process could represent risk factors, which had not been accounted for as yet.

A poly(ADP-ribose) polymerase haplotype spanning the promoter region confers susceptibility to rheumatoid arthritis

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Objective. To investigate the association of the poly(ADP-ribose) polymerase (PARP) gene promoter polymorphism in relation to rheumatoid arthritis (RA) predisposition.

Methods. An association study comprising 213 Spanish RA patients and 242 healthy subjects was carried out to investigate the association of all known PARP gene promoter polymorphisms with disease susceptibility, which includes a CA microsatellite repeat, a poly(A)n and 3 single point mutations, namely C410T, C1362T and G1672A. Additionally, we analysed the distribution of PARP polymorphisms in 58 Spanish families with one or more affected members.

Results. After complete genotyping of our panel of 455 samples, strong linkage disequilibrium (LD) was observed among the 5 PARP polymorphisms. Only 2 PARP haplotypes were detected: haplotype "A": 410T-(A)10-(CA)10-12-1362C that includes short PARP CA alleles and haplotype "B": 410C-(A)11-(CA)13-20-1362T, always paired with long PARP CA variants. Regarding the G1672A variation, although LD was detected it does not seem to be part of the conserved haplotypes described. Haplotype B was statistically overrepresented in the RA patient group compared with the healthy subjects ($P = 0.019$ OR 1.42, 95% CI 1.06-1.91). In addition, a significant dose effect of PARP haplotype carriage on disease predisposition could be observed. Of note, within haplotype B, the PARP CA 97 bp allele was found to be the RA-predisposing marker ($P = 0.003$, $Pc < 0.05$ OR 2.17, 95% CI 1.27-3.72.).

Conclusion. Our results describe the existence of 2 unique PARP haplotypes in the Spanish population and provide the first evidence that PARP haplotypes play a role on susceptibility to RA.

Protective role of the antioxidant proteins metallothioneins in experimental autoimmune encephalomyelitis, animal model of multiple sclerosis

Eva M. Martínez-Cáceres, Milena Penkowa, Carmen Espejo, Xavier Montalban, Juan Hidalgo

Methallotioneins (MT) are a family of low molecular weight, heavy metal-binding, cisteine-rich proteins that accumulate under conditions where oxidative stress has taken place and they may provide protection against oxygen radicals and oxidative damage caused by inflammation, tissue injury and stress (1).

We show that MT are induced in inflammatory infiltrates during experimental autoimmune encephalomyelitis (EAE) and multiple sclerosis (MS) (2,3).

Further studies in EAE demonstrated that susceptibility to the disease is higher in MT deficient mice (MTKO) (4) and that demyelination and axonal damage are significantly increased in MTKO mice compared to control animals (5). These data together with the proof that treatment with MT improves EAE (6) support a neuroprotective role for these proteins in the CNS and suggest that MT treatment may well become a therapeutic approach for MS in the future.

References:

(1) Hidalgo J, Aschner M, Zatta P, Vasak M Role of the metallothionein family of proteins in the central nervous system. Brain Res. Bull. 2001; 55: 133-145

(2) Espejo C, Carrasco J, Hidalgo J, Penkowa M, Garcia A, Sáez-Torres I, Martínez-Cáceres EM. Metallothionein induction in the central nervous system of mice with experimental autoimmune encephalomyelitis Neuroscience 2001; 105: 1055-65

(3) Penkowa M, Espejo C, Ortega A, Hidalgo J, Montalban X, Martínez-Cáceres EM. Metallothionein expression in the central nervous system of multiples sclerosis patients Submitted for publication

(4) Penkowa M, Espejo C, Martínez-Cáceres EM, Bjørn Poulsen C, Montalban X, Hidalgo J. Altered inflammatory response and increased neurodegeneration in metallothionein I+II deficient mice during experimental autoimmune encephalomyelitis J Neuroimmunol 2001; 119: 248: 60

(5) Penkowa M, Espejo C, Martínez-Cáceres EM, Montalban X, Hidalgo J Increased demyelination and axonal damage in metallothionein I+II deficient mice during experimental autoimmune encephalomyelitis Cel Mol Life Sci 2003 (en prensa)

(6) Penkowa M and Hidalgo J Metallothionein I+ II (MT I+II) expression and their role on experimental autoimmune encephalomyelitis (EAE). Glia 2000; 32: 247-263

Resistance to superantigen-induced deletion among NOD T cells in embryo aggregation mouse chimeras

Vinicius Motta, Kristina Lejon, Dan Holmberg

Although the development of autoimmune disease in nonobese diabetic mice (NOD) has been mostly attributed to defects in peripheral tolerance mechanisms, recent data have suggested that NOD mice display a defect in negative selection of thymocytes. Here, we track the fate of Vb11 from NOD and C3H origins in embryo aggregation mouse chimeras (EA).

EA consists of aggregating embryos at 2.5 days in vitro, transferring to a foster mother, which gives birth to chimeric mice. Since endogenous superantigens presented in C3H delete Vb11 thymocytes, we quantified the percentage of NOD Vb11 and C3H Vb11 cells in our chimeras. We show that higher percentages of NOD Vb11 is found in our chimeras when compared to C3H Vb11. Deletion of Vb11 in C3H wild type mice occurs at the transition of double positive (DP) to single positive (SP) stage during the thymocyte development, mainly at the semi-mature stage (single positives HSAhi). We also show that anti-CD3-induced apoptosis in DP thymocytes occurs at similar levels between NOD and C3H thymocytes in fetal thymic organ culture (FTOC). Thus, our data supports previous data showing that NOD mice display a defect in negative selection, which seems to be a consequence of semi-mature thymocytes to resist apoptosis induction.

Constitutive expression of AID leads to tumorigenesis

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Genome stability is regulated by the balance between efficiencies of the repair machinery and genetic alterations such as mutations and chromosomal rearrangements. It has been postulated that deregulation of class siwtch recombination (CSR) and somatic hypermutation (SHM), which modify the immunoglobulin (Ig) genes in activated B cells, may be responsible for aberrant chromosomal translocations and mutations of non-Ig genes that lead to lymphocyte malignancy. However, the molecular basis for these genetic instabilities is not clearly understood. Activation-Induced cytidine Deaminase (AID) is shown to be essential and sufficient to induce both CSR and SHM in artificial substrates in fibroblasts as well as B cells. Here we show that constitutive and ubiquitous expression of AID in transgenic mice caused both T cell lymphomas and dysgenetic lesions of epithelium of respiratory bronchioles (micro-adenomas) in all individual mice. Point mutations, but not translocations, were massively introduced in expressed T cell receptor (*TCR*) and c-*myc* genes in T lymphoma cells. The results indicate that AID can mutate non-Ig genes including oncogenes, implying that aberrant AID expression could be a cause of human malignancy.

Autoantibodies against cardiac Troponin I are responsible for the dilated cardiomyopathy in PD-1 deficient mice

Taku Okazaki, Yoshimasa Tanaka, Ryosuke Nishio, Tamotsu Mitsuiye, Akira Mizoguchi, Akira Matsumori, Nagahiro Minato, and Tasuku Honjo

PD-1 is an immuno-inhibitory receptor and belongs to the CD28/CTLA-4 family. PD-1 deficient mice develop various autoimmune diseases depending on their genetic background; C57BL/6-PD-1 deficient mice develop lupus-like glomerulonephritis and arthritis, and BALB/c-PD-1 deficient mice develop autoimmune dilated cardiomyopathy with high-titered autoantibodies against a heart specific 30kDa protein. We have purified this 30kDa protein from heart extract and identified it as cardiac Troponin I. Monoclonal antibodies against cardiac Troponin I stained the surface of cardiomyocytes, and augmented the voltage dependent calcium current of normal cardiomyocytes. Furthermore, administration of monoclonal antibodies against cardiac Troponin I induced dilatation and dysfunction of heart in wild type mice. These findings demonstrate the pathogenic role of anti-cardiac Troponin I autoantibodies in the dilated cardiomyopathy in PD-1 deficient mice.

CD69 down-regulates immune reactivity through active TGF-beta production in collagen-induced arthritis

David Sancho, Manuel Gómez, Fernando Viedma, Enric Esplugues, Mónica Gordón-Alonso, María Angeles García-López, Carlos Martínez-A, Pilar Lauzurica, Francisco Sánchez-Madrid

CD69 is induced following activation of leukocytes at inflammatory sites, but its physiological role remains unknown. We explored the role of CD69 in the immune reactivity by analyzing a model of collagen-induced arthritis (CIA) in wild type and CD69-deficient mice. $CD69^{-/-}$ mice showed higher incidence and severity of CIA, with exacerbated immune response to type II collagen. TGF-beta1, which is protective in CIA, was reduced in $CD69^{-/-}$ mice inflammatory foci. Local injection of blocking anti-TGF-beta increased CIA severity in $CD69^{+/+}$, but not in $CD69^{-/-}$ mice. Moreover, *in vitro* engagement of CD69 induced TGF-beta1 production both in mouse and human synovial leukocytes. Our results unveil CD69 as a negative modulator of immune reactivity and inflammation through TGF-beta synthesis that regulates other pro-inflammatory cytokines, such as IL-1beta or RANTES.

The role of CD69 as a negative modulator of immune reactivity opens the possibility of an interesting and novel approach for the therapy of chronic inflammation and other immune-mediated diseases by targeting CD69.

In addition, since CD69 is selectively expressed in activated leukocytes infiltrating inflamed tissues, the pharmacological stimulation of TGF-beta synthesis through CD69 may have a localized effect, thus avoiding the detrimental consequences of a systemic TGF-beta up-regulation. As for other regulatory molecules (e.g., CTLA-4), CD69 could be an interesting target for the therapy of immune-mediated diseases.

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38 **Workshop on Immunodeficiencies of Genetic Origin.**
Organizers: A. Fischer and A. Arnaiz-Villena.

39 **Workshop on Molecular Basis for Biodegradation of Pollutants.**
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*40 **Workshop on Nuclear Oncogenes and Transcription Factors in Hematopoietic Cells.**
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*41 **Workshop on Three-Dimensional Structure of Biological Macromolecules.**
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42 **Workshop on Structure, Function and Controls in Microbial Division.**
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*43 **Workshop on Molecular Biology and Pathophysiology of Nitric Oxide.**
Organizers: S. Lamas and T. Michel.

*44 **Workshop on Selective Gene Activation by Cell Type Specific Transcription Factors.**
Organizers: M. Karin, R. Di Lauro, P. Santisteban and J. L. Castrillo.

45 **Workshop on NK Cell Receptors and Recognition of the Major Histocompatibility Complex Antigens.**
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46 **Workshop on Molecular Mechanisms Involved in Epithelial Cell Differentiation.**
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48 **Workshop on G-Proteins: Structural Features and Their Involvement in the Regulation of Cell Growth.**
Organizers: B. F. C. Clark and J. C. Lacal.

*49 **Workshop on Transcriptional Regulation at a Distance.**
Organizers: W. Schaffner, V. de Lorenzo and J. Pérez-Martín.

50 **Workshop on From Transcript to Protein: mRNA Processing, Transport and Translation.**
Organizers: I. W. Mattaj, J. Ortín and J. Valcárcel.

51 **Workshop on Mechanisms of Expression and Function of MHC Class II Molecules.**
Organizers: B. Mach and A. Celada.

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Organizers: R. W. Oppenheim, E. M. Johnson and J. X. Comella.

57 **Workshop on NF-κB/IκB Proteins. Their Role in Cell Growth, Differentiation and Development.**
Organizers: R. Bravo and P. S. Lazo.

58 **Workshop on Chromosome Behaviour: The Structure and Function of Telomeres and Centromeres.**
Organizers: B. J. Trask, C. Tyler-Smith, F. Azorín and A. Villasante.

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60 **Workshop on Abscisic Acid Signal Transduction in Plants.**
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63 **Workshop on TGF-β Signalling in Development and Cell Cycle Control.**
Organizers: J. Massagué and C. Bernabéu.

64 **Workshop on Novel Biocatalysts.**
Organizers: S. J. Benkovic and A. Ballesteros.

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66 **Workshop on 100th Meeting: Biology at the Edge of the Next Century.**
Organizer: Centre for International Meetings on Biology, Madrid.

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Organizers: V. Malhotra and A. Velasco.

68 **Workshop on DNA Repair and Genome Instability.**
Organizers: T. Lindahl and C. Pueyo.

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Organizers: J. L. R. Arrondo, F. M. Goñi, B. De Kruijff and B. A. Wallace.

80 **Workshop on Plasmodesmata and Transport of Plant Viruses and Plant Macromolecules.**
Organizers: F. García-Arenal, K. J. Oparka and P.Palukaitis.

81 **Workshop on Cellular Regulatory Mechanisms: Choices, Time and Space.**
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82 **Workshop on Wiring the Brain: Mechanisms that Control the Generation of Neural Specificity.**
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83 **Workshop on Bacterial Transcription Factors Involved in Global Regulation.**
Organizers: A. Ishihama, R. Kolter and M. Vicente.

84 **Workshop on Nitric Oxide: From Discovery to the Clinic.**
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85 **Workshop on Chromatin and DNA Modification: Plant Gene Expression and Silencing.**
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86 **Workshop on Transcription Factors in Lymphocyte Development and Function.**
Organizers: J. M. Redondo, P. Matthias and S. Pettersson.

87 **Workshop on Novel Approaches to Study Plant Growth Factors.**
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91 **Workshop on Eukaryotic Antibiotic Peptides.**
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107 **Workshop on Biochemistry and Molecular Biology of Gibberellins.**
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143 **Workshop on Engineering RNA Virus Genomes as Biosafe Vectors.**
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148 **Workshop on Membranes, Trafficking and Signalling during Animal Development.**
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Organizers: P. Levitt, D. A. Lewis and J. DeFelipe.

150 **Workshop on Plasticity in Plant Morphogenesis.**
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151 **Workshop on Wnt Genes and Wnt Signalling**
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