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SCI
SOCIETAT CATALANA D'IMMUNOLOGIA

VIII CONGRÉS

Societat Catalana d'Immunologia (SCI)

Programa Final

Barcelona, 20 i 21 de novembre de 2014

Microbiota i immunitat de les mucoses

Microbiota and mucosal immunity

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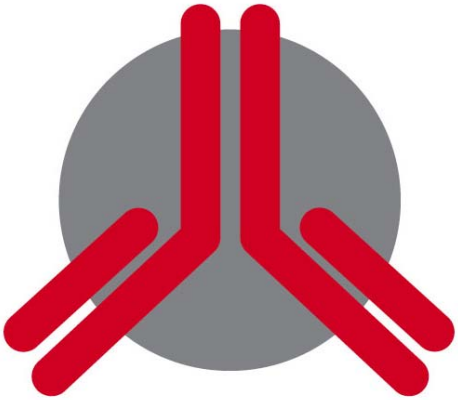

Congress Office:
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Welcome to the Congress,

On behalf of the organising committee, we would like to warmly welcome you to the VIIIth Societat Catalana d'Immunologia (SCI) Congress. We believe that our meeting will present high level scientific knowledge with the contribution of immunologists and specialists who are experts in this field.

Dr. Cándido Juárez
SCI President

VIIIth Congress of the Catalan Society of Immunology: Microbiota and mucosal immunity, has been accredited by the Catalan Lifelong Learning Board of the Healthcare Professions with 1 credit (ref 09 / MD-11448, 11/12/2014).

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Content

Scheme first day

Thursday, November 20th	
15:30 17:30	Arrival, Registration and Documentation delivery
16:20 16:30	**Welcome to the VIIIth CONGRESS of SCI** Dr. Cándido Juárez (President of SCI)
16:30 17:30	Chair: Dr. Cándido Juárez (S. Immunologia, HSCSP) **DR. FRANCISCO GUARNER** Servei Aparell Digestiu. Hospital Vall d'Hebron. Barcelona ***"Impact of microbiota in health"***
17:30 17:45	Poster viewing – Coffee Break *Posters can be viewed on the 4 electronic panels located in the Hall*
17:45 18:45	Chair: Dra. María José Amengual (S. Immunologia, Corp. Sanitaria Parc Taulí) **DR. LEONIDES FERNÁNDEZ ÁLVAREZ** Departamento de Nutrición, Bromatología y Tecnología de los Alimentos de la Universidad Complutense de Madrid. Madrid ***"Revealing the importance of human milk microbiota"***
18:45 19:45	Chair: Dr. Carles Morte (Kymos Pharma Services) **DR. CARLOS UBEDA** Departamento de Genómica y Salud, Centro Superior de Investigación en Salud Pública–FISABIO. Valencia ***"The dark side of antibiotics: changes on the microbiota enable infection by antibiotic resistant pathogens"***
19:50	**End of session**

Scheme second day

Friday, November 21th	
08:30 08:55	Arrival, Registration and Documentation delivery
09:00 10:00	Chair: Dr. Francisco José Pérez-Cano (Fac. Farmàcia, UB) **DR. PEER BRANDTZAEG** Department of Pathology, Oslo University Hospital, Rikshospitalet, Oslo, Norway. ***"The mucosal IgA system: functions and enigmas"***
10:00 11:00	Oral Communications Session I: **Innate Immunity** Chair: Dr. Jorge Lloberas (UB) and Dr. Francisco José Pérez-Cano (UB) 10:00h Antigen-specific myeloid-derived suppressor cells ameliorate experimental autoimmune encephalomyelitis. **Sílvia Casacuberta-Serra** et al. (oral presentation 1). 10:12h Prostaglandin E2 receptor (EP2) is involved in supressive function of human tolerogenic dendritic cells. **Georgina Flórez-Grau** et al. (oral presentation 2). 10:24h Hyperosmotic stress control gene expression on macrophages. **Eros Marín** et al. (oral presentation 3). 10:36h Vsl#3 probiotic treatment decreases bacterial translocation in rats with carbon tetrachloride-induced cirrhosis. **Juan Nieto** et al. (oral presentation 4). 10:48h Control of tumor growth by Ly6C^{high} monocytes. **Fatemeh Zar**e et al. (oral presentation 5).
11:00 11:30	Poster viewing – Coffee Break *Posters can be viewed on the 4 electronic panels located in the Hall*
11:30 12:30	Chair: Dr. Daniel Benítez (CIBERehd, Hospital Clínic) **DR. MARIA RESCIGNO** Dept. of Experimental Oncology. European Institute of Oncology, Milan, Italy ***"Microbiota in gut homeostasis and cancer"***
12:30 13:30	**Assemblea General Ordinària** **SOCIETAT CATALANA d'IMMUNOLOGIA** (12.30h – First Call) *Us hi esperem a tots: els socis i no-socis!!*
13:30 15:00	Poster viewing – LUNCH *Posters can be viewed on the 4 electronic panels located in the Hall*

15:00 16:00	Chair: Dr. Ramón Gimeno (IMIM) **DR. TOM CUPEDO** Department of Hematology, Erasmus University Medical Center, Rotterdam, The Netherlands ***"Innate lymphoid cells"***
16:00 16:55	Oral Communications Session II: **Clinical Immunology** Chair: Dr. Ramón Gimeno (IMIM) and Dra. María José Amengual (CSPT) 16:00h First report of somatic NOD2 mosaicism in Blau syndrome. **Anna Mensa-Vilaró** et al. (oral presentation 6). 16:12h Dissecting the role of the intestinal epithelial barrier in the pathogenesis of ulcerative colitis. **Isabella Dotti** et al. (oral presentation 7) 16:24h A novel missense mutation in FOXP3 affecting the position F367 in a family with IPEX syndrome illustrates the complexity of in silico pathogenicity prediction. **Roger Colobran** et al (oral presentation 8) 16:36h Aplicación clínica del cribado genotípico del C4 para el seguimiento personalizado de pacientes con LES. **Rebeca Sánchez Hidalgo** et al. (oral presentation 9) 16:48h Diagnostic of Familial Hemophagocytic Lymphohistiocytosis type 5 (FHL5) caused by mutations in STXBP2 gene. **Laura Viñas** et al. (oral presentation 10)
16:55 17:20	Poster viewing – Coffee Break *Posters can be viewed on the 4 electronic panels located in the Hall*
17:20 18:15	Oral Communications Session III: **Adaptative Immunity** Chair: Dra. Mercè Martí (UAB) and Dra. Eva M. Martínez-Cáceres (HUGTiP) 17:20h Mucosal Immunity in fish: learning from lower vertebrates. **David Parra** et al. (oral presentation 11) 17:32h Gut Microbial Antigen Specific $CD4^{+}$T cells from Crohn's Disease patients exhibit a pro-inflammatory Th17 Phenotype. **Elisabeth Calderón-Gómez** et al. (oral presentation 12) 17:44h Efecte modulador de la deficiència de CD6 davant l'estimulació al·logènica. **Marta Consuegra-Fernández** et al. (oral presentation 13) 17:56h A founder splicing mutation in CD3δ in a $T\alpha\beta^{-}T\gamma\delta^{+}B^{+}NK^{+}$ SCID pedigree of ecuadorian descent**. Laura Martínez-Martínez** et al. (oral presentation 14) 18:08h Molecular and phenotypical analysis of transgenic mice for soluble human CD6 expression. **Inês Simões** et al. (oral presentation 15)
18:15 19:15	Chair: Dra. Mercè Martí (Fac. Biociències, UAB) **DR. ANDREA CERUTTI** Institut Hospital del Mar d'Investigacions Mèdiques (IMIM), Barcelona Biomedical Research Park and The Immunology Institute, Department of Medicine, Mount Sinai School of Medicine, New York. ***"Mucosal Immunology"***
19:15 19:30	**Prize to the best communication and poster in the Congress.** **Dr. Cándido Juárez (President of SCI)** **End of Congress**

Corporative Abbreviations

BST: Banc de Sang i Teixits
CEMCAT: Centre d'Esclerosi Múltiple de Catalunya
CIBER: Centro de Investigación Biomédica en Red
CIBERehd: Centro de Investigación Biomédica en Red de Enfermedades Hepáticas y Digestivas
CRC: Centre de Recherche des Cordeliers
CSPT: Corporació Sanitaria Parc Taulí
HIVACAT: Institut de Recerca de la Sida IrsiCaixa i el Servei de Malalties Infeccioses i Sida de l'Hospital Clínic de Barcelona
HUGTiP: Hospital Universitari Germans Trias i Pujol
HSCSP: Hospital de la Santa Creu i Sant Pau
HUVH: Hospital Universitari Vall d'Hebron
IDIBAPS: Institut d'Investigacions Biomèdiques August Pi i Sunyer
IIB: Institut d´Investigacions Biomèdiques Sant Pau
IGTP: Institut d'Investigació en Ciències de la Salut Germans Trias i Pujol
IMIM: Institut Hospital del Mar d'Investigacions Mèdiques
INSA: Institut de Recerca en Nutrició i Seguretat Alimentària
IRB Barcelona: Institut de Recerca Biomèdica Barcelona
IRB Lleida: Institut de Recerca Biomèdica Lleida
PCB: Parc Científic de Barcelona
PRBB : Parc de Recerca Biomèdica de Barcelona
SCI: Societat Catalana d'Immunologia
UAB: Universitat Autònoma de Barcelona
UB: Universitat de Barcelona
UPF: Universitat Pompeu Fabra
VHIR: Vall d'Hebron Institut de Recerca

Abstracts

Oral Communications Session I
Innate Immunity 1 - 5

1 Antigen-specific myeloid-derived suppressor cells ameliorate experimental autoimmune encephalomyelitis.

Sílvia Casacuberta-Serra[1,3]; Carme Costa[2,3,4]; Herena Eixarch[2,3,4]; Sergio López-Estévez[1,3]; Lluís Martorell[1,3]; Xavier Montalban[2,3,4]; Carmen Espejo[2,3,4,5]; Jordi Barquinero[1,3,5].

[1]Laboratori de Teràpia Gènica i Cel·lular, Vall d'Hebron Institut de Recerca (VHIR); [2]Servei de Neurologia-Neuroimmunologia. Centre d'Esclerosi Múltiple de Catalunya (Cemcat); [3]Universitat Autònoma de Barcelona; [4]Vall d'Hebron Institut de Recerca (VHIR); [5]These authors contributed equally to this work.

We previously reported that the transfer of bone marrow cells (BMCs) transduced with MOG40-55 peptide into mice with experimental autoimmune encephalomyelitis (EAE) improved the disease. We also showed that the majority of cells generated in standard retroviral transduction cultures of BMCs consist of transgene-expressing myeloid-derived suppressor cells (MDSCs). The present work was aimed at characterizing these MDSCs generated *ex vivo* and investigating their contribution to the therapeutic effect observed. To this end, we transduced BMCs with either the retroviral vector encoding the autoantigen (IiMOG) or a control vector (Ii). A single infusion of transduced total BMCs or purified MDSCs was administered seven days before (preventive arm) or 13 days after (therapeutic arm) EAE induction. Mice were daily assessed for clinical signs using a 6-point scale. In the preventive approach, the infusion of BM- and MDSC-IiMOG cells but not their controls ameliorated the disease, indicating that antigen-specific MDSCs likely contribute to the therapeutic effect. Furthermore, MDSCs-IiMOG treated mice presented reduced percentages of activated T cells ($CD3^+CD4^+CD25^+FoxP3^-$) and increased B regulatory cells ($CD45^+B220^+CD5^+CD1d^+$) in the spleens compared to controls. Therapeutic infusion of BM- and MDSCs-IiMOG cells was associated with a significantly improvement of accumulated clinical scores. In the preventive approach, mice treated with MOG-specific BMCs or MDSCs showed milder inflammatory infiltration and demyelination in the spinal cord. Both IiMOG treated groups showed significantly less T cell infiltration, microglia activation, astrogliosis, axonal damage and demyelination. In the therapeutic approach, mice treated with BM-IiMOG also presented a significant reduction in less T cell infiltration, microglia activation, astrogliosis, axonal damage and demyelination in the spinal cord. Histopathological studies for mice treated with MDSCs are currently ongoing. In summary, retroviral transduction of murine hematopoietic cells generates antigen-specific MDSCs that have a therapeutic effect in EAE.

2 Prostaglandin E2 receptor (EP_2) is involved in supressive function of human tolerogenic dendritic cells

Georgina Flórez-Grau[1,3,4]; Raquel Cabezón[1,3,4]; Carolina España[3,4]; Julián Panés[1,3]; Daniel Benítez-Ribas[2,3,4].

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Dendritic cells (DCs) are considered one of the most important antigen-presenting cells and have a crucial role in linking innate and adaptive immune responses.

In the last decade, these cells have been used in medicine to modify the immune response in several diseases that range from cancer to autoimmunity. To inhibit inflammatory responses, dexamethasone (dex) has been broadly used as a tolerogenic agent, due to its efficacy in suppressing maturation of DCs. Several reports have characterized dexamethasone-induced tolerogenic DCs (tol-DCs) showing increased of IL-10 production and their ability to promote Treg induction.However these tol-DCs need further molecular characterization that would help us to better understand and define the molecules and mechanisms involved in tolerance induction.

Based on previous data from our lab, Prostaglandin E2 receptors (EP) were selected for further studies. During the in vitro generation of tol-DCs, Prostaglandin E2 (PGE_2) is often used as a part of maturation cocktail (MC). PGE_2 has versatile functions in the organism. Furthermore it plays an important role as an immune regulator, both activating and inhibiting immune responses. PGE_2 is able to exert multiple functions through four different receptors, designed as EP receptors (1-4). We herein describe how PGE_2 receptors in human DCs are differently regulated by dex having functional consequences.

In order to investigate the differential effect of PGE_2 in tol-DCs versus mature dendritic cells (mDCs) we analyzed the expression of EP receptors. $EP_{2,\ 3,\ 4}$ receptors were up-regulated in both tol-DCs and mDCs at both mRNA and protein levels relative to immature dendritic cells (iDCs).

Moreover, we observed that PGE_2 has an important role in regulation of IL-10 production by tol-DCs. When PGE_2 is absent in MC, IL-10 production by tol-DCs is highly reduced in response to LPS and *E. coli*.

To study which EP receptor was involved in IL-10 production we treated DCs with specific agonists of each receptor (2 and 4).Supernatants of tol-DCs treated with EP agonists were tested for IL-10 production showing that EP2 specific agonist was able to induce high levels of IL-10 respecting to PGE2 –treated tol-DCs. In contrast EP4 agonist inhibited IL-10 production by tolDCs.

To confirm these results we performed experiments adding EP_2 and EP_4 receptor antagonists. Interestingly, neutralization of EP_2 receptor inhibited IL-10 production suggesting that EP_2, but not EP_4, is directly involved in the regulation of IL-10 in tolDCs.

In conclusion, these findings show that dex induces changes inregulates the expression PGE_2 receptors in human DCs. In particular, $EP_{2,3,4}$ were up-regulated in tol-DCs compared to mDCs. Interestingly, we describe that PGE_2 acts in tol-DCs through EP_2, which is related with the anti-inflammatory role of PGE_2 and in concordance with the anti-inflammatory environment around tol-DCs generated with dex. We can conclude that EP_2 is the prostaglandin receptor involved in tolerance.

3 Hyperosmotic stress control gene expression on macrophages.

Eros Marín; Joan Tur; Antonio Celada; Jorge Lloberas.

Dept. Fisiologia i Immunologia, Fac. Biologia, Parc Cientific de Barcelona, Universitat de Barcelona.

Hyperosmotic stress is linked to many diseases, as well as local or systemic inflammatory disorders. The osmolarity of plasma (110mM) is the consequence of the local presence of ions. The variations that occur in situations as hipertonicity or hipotonicity have different effects depending the kind of tissues. Previous studies have shown that high concentrations of NaCl (200mM) decrease the inflammatory response in neutrophils and macrophages because it decreases the inflammatory cytokines production. In this work we investigate the effects of hyperosmotic stress in macrophages stimulates with the differentiation factor GM-CSF, the pro-inflammatory molecule LPS or the anti-inflammatory citokine IL-4. In GM-CSF-stimulated macrophages, in the presence of hyperosmotic stress, we saw decreased expression of differentiations genes irf2 and irf4 as well as genes involved in proliferation c-myc and ccnd1 without changes in STAT5a phosphorylation pattern. In LPS-stimulated macrophages, in a moderate-hyperosmotic stress condition (155mM of NaCl), we observed an increased production of nitric oxide, oxygen reactive species and il-1b and il-12 citokines expression. However, no modifications were observed in a several-hiperosmotic stress condition (200mM of NaCl). Both concentrations of NaCl in LPS-stimulated macrophages induce changes in the kinetics of phosphorylation in MAPKs, ERK1/2 and JNK. Finally, in IL-4-stimulated macrophages we observed an increased expression of arg-1. In conclusion, gene expression of macrophages is affected by the hyperosmotic stress.

4 Vsl#3 probiotic treatment decreases bacterial translocation in rats with carbon tetrachloride-induced cirrhosis

Juan Camilo Nieto[2,6,9]*, Elisabet Sánchez[1,6,8,9]*; Ana Boullosa[7]; Silvia Vidal [2,6,9]; Francesc Josep Sancho[4]; Giacomo Rossi[10]; Pau Sancho-Bru[7,8]; Rosa Oms[5]; Beatriz Mirelis[3,9]; Cándido Juárez[2,9]; Carlos Guarner[1,6,8,9]; Germán Soriano[1,6,8,9].

[1]Department of Gastroenterology, [2]Department of Immunology, [3]Department of Microbiology, [4]Department of Pathology; [5]Department of Biochemistry, Hospital de la Santa Creu i Sant Pau, Barcelona, Spain. [6]Institut d´Investigacions Biomèdiques (IIB) Sant Pau, Barcelona, Spain. [7]Institut Investigacions Biomèdiques August Pi i Sunyer (IDIBAPS), Hospital Clínic de Barcelona, Barcelona, Spain. [8]Centro de Investigación Biomédica en Red de Enfermedades Hepáticas y Digestivas (CIBERehd), Instituto de Salud Carlos III, Madrid, Spain; [9]Universitat Autònoma de Barcelona, Bellaterra (Cerdanyola del Vallès), Spain. [10]School of Veterinary Medical Sciences, University of Camerino, Camerino, Italy

Background. Probiotics can prevent pathological bacterial translocation in cirrhosis by modulating intestinal microbiota and improving gut barrier and immune disturbances.

Aim: To evaluate the effect of probiotic VSL#3 on bacterial translocation, intestinal microbiota, gut barrier and inflammatory response in rats with experimental cirrhosis.

Methods: Forty-six Sprague-Dawley rats with CCl4-induced cirrhosis were randomized into two groups: VSL#3 group (n=22) that received VSL#3 in drinking water, and water group (n=24) that received water only. Treatment began at week 6 of cirrhosis induction and continued until laparotomy, performed one week after development of ascites or at week 20. A control group included 11 healthy rats. At the end of the study we evaluated bacterial translocation, intestinal flora, intestinal barrier (ileal claudin-2 and 4, β-defensin-1, occludin and malondialdehyde as index of oxidative damage) and serum cytokines.

Results: Mortality during the study was similar in the VSL#3 group (10/22, 45%) and the water group (10/24, 42%) (p=1). The incidence of bacterial translocation was 1/12 (8%) in the VSL#3 group, 7/14 (50%) in the water group 2 (p=0.03 vs VSL#3 group) and 0/11 in the control group (p=0.008 vs water group). The concentration of ileal and cecal enterobacteria and enterococci was similar in the two groups of cirrhotic rats. The ileal occludin concentration was higher and ileal malondialdehyde and serum levels of TNF-α were lower in the VSL#3 group than in the water group (p<0.05).

Conclusions: VSL#3 decreases bacterial translocation and improves the intestinal barrier and proinflammatory state in rats with experimental cirrhosis.

5 Control of tumor growth by Ly6C^{high} monocytes

Fatemeh Zare; Juan Antonio Calatayud; Luis F. Santamaria; Antonio Celada; Jorge Lloberas

Dept. Fisiologia i Immunologia, Fac. Biologia, Parc Cientific de Barcelona, Universitat de Barcelona

Cancer immunotherapy represents a treatment strategy which is being clinically tested to complement surgery, radiotherapy and chemotherapy – the current cornerstones of our fight against cancer. It has become clear now, that tumors not only escape immune recognition but also actively suppress antitumor immune responses. In order to improve cancer immunotherapy, effective manipulation of the immune system to break self-tolerance need to be designed and approaches that counteract immunosuppressive mechanisms need to be developed. The tumor microenvironment encompasses a wide variety of immune cells, which macrophages comprise the most dominant portion of them and thus are the major players in the connection between inflammation and cancer. These tumor-associated macrophages (TAMs) are derived from blood monocyte precursors and subsequently acquire distinct characteristics as a result of tumor micro-environmental cues. Monocytes are a heterogeneous population in the blood with an enormous plasticity whose fate and functions are dictated by the microenvironment. Therefore, the roles of specific Monocytes subsets in tumor progression and the molecular mechanisms for their impacts need to be elucidated. Ly6C^{high} and Ly6C^{low} are two main different types of murine monocytes subsets that have been defined by distinct phenotypes and immunoregulatory functions. Recent data demonstrates that Ly6C^{high} monocytes can recruit to inflammation loci while Ly6C^{low} monocytes are patrolling cells. We have developed a method to produce large number of Ly6C^{high} monocytes in vitro. In our study we observed that, injection of these cells affects tumor progression in breast cancer and C26 colon carcinoma. Activation of Ly6C^{low} monocytes by pro- or anti-inflammatory cytokines, results in a genetic expression profile, corresponding to pro- or anti-inflammatory genes, respectively. Injection of pre- activated Ly6C^{low} monocytes toward pro- or anti-inflammatory polarization in C26 colon carcinoma showed that anti-inflammatory activated monocytes are more beneficial in delaying cancer cachexia. Increased knowledge of monocytes improves the chances to find therapies against a broad spectrum of diseases including cancer, where monocytes have opposing roles of either being beneficial or detrimental to the host.

6 First report of somatic NOD2 mosaicism in Blau syndrome

Anna Mensa-Vilaró[1]; Eva González-Roca[1]; Jaime De Inocencio[3]; Pilar Tejada Palacios[4]; Eugenia Enriquez-Merayo[3]; Giuliana Magri[4]; Andrea Cerutti[4]; Susana Plaza[1]; María Carmen Antón[1]; Estíbaliz Ruiz-Ortiz[1]; Jordi Yagüe[1]; Juan I. Aróstegui[1].

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Introduction: Blau syndrome (BS) is a rare dominantly inherited autoinflammatory disease characterized by granulomatous arthritis, dermatitis and uveitis. Genetic studies have shown that it is caused by *gain-of-function* mutations in the *NOD2* gene, which encodes the cytosolic Nod2 protein. Somatic gene mosaicism has been well established in cancer. In recent years, this genetic mechanism has also been described in different Mendelian inherited diseases such as autoimmune lymphoproliferative syndromes, cryopyrinopathies and STING-associated vasculopathy with onset in infancy. Here we describe for the first time the involvement of somatic *NOD2* mosaicism in BS pathogenesis.

Objectives: To evaluate the presence of somatic *NOD2* mosaicism in a patient with a clinical diagnosis of BS but mutation negative by Sanger sequencing.

Material and Methods: Patient's data were collected through charts' review and direct interviews. Genomic DNA was extracted from different haematological and non-hematological tissues. *NOD2* analysis was performed by Sanger sequencing and afterwards by targeted deep sequencing.

Results: Sequencing of *NOD2* by Sanger method did not detect any disease-causing mutation. However, a careful analysis of chromatograms revealed a potential c.1001G>A transition, which might provoke the appearance of the p.Arg334Gln *NOD2* variant. This variant is a well-known BS-causing mutation, and we hypothesized the presence of somatic *NOD2* mosaicism. Targeted deep *NOD2* sequencing confirmed our hypothesis by means of the detection of the p.Arg334Gln mutation in all analyzed samples, with variable degree of somatic mosaicism (4.9-11.0%) depending on the cell's origin. The absence of the *NOD2* mutation in patient's parents supported its *de novo* nature. Comparative phenotype analysis with BS patients carrying germline *NOD2* mutations revealed a mild articular involvement and absence of extra-triad symptoms in patient with somatic mosaicism.

Conclusions: Herein we report for the first time the involvement of somatic *NOD2* mosaicism in the pathogenesis of BS. Our data provide new insights into the role of low-level mosaicism as the pathophysiologic mechanism underlying Mendelian inherited diseases. The use of next-generation sequencing technologies allows quick and accurate investigation of gene mosaicism, which may have significant clinical consequences.

7 Dissecting the role of the intestinal epithelial barrier in the pathogenesis of ulcerative colitis

Isabella Dotti[1]; Núria Planell[1,3]; Peter Jung[2]; Eduard Batlle[2]; Julián Panés[1]; Azucena Salas[1].

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Ulcerative colitis (UC) is a chronic inflammatory bowel disease (IBD) of unknown aetiology. Alterations in the colonic epithelial barrier contribute to the onset of UC. However, little data is available about the primary role of epithelial cell (EC) dysfunction in this disease. Recently, a novel cell-culture system has been established to study gastrointestinal EC physiopathology.

Using this approach we aim to investigate whether a primary defect in intestinal EC function is present in UC patients that could drive the development of an inflammatory response.

To this end, we collected biopsies from the sigmoid colons of 6 UC patients and 4 non-IBD controls. Isolated crypts were cultured and stem ECs were expanded in Matrigel as “organoids”. Total RNA from stem and differentiated organoids was extracted for transcriptional analysis.

Our results show that control and UC organoids follow common differentiation programs with comparable downregulation of stem and proliferation markers (i.e., Lgr5, Ki67) and upregulation of EC differentiation markers (i.e., MUC2, ANPEP, ChrA). However, statistical analysis of microarrays revealed more than 800 genes differentially expressed between control and UC organoids, with cellular movement and development, lipid metabolism, and molecule transport the most significantly altered cellular functions.

In the present study the organoid culture model has been successfully used to detect intrinsic differences in the epithelium of UC patients. Validation experiments are ongoing to confirm our results in an independent cohort of patients and to dissect the functional meaning of such alterations.

8 A novel missense mutation in FOXP3 affecting the position F367 in a family with IPEX syndrome illustrates the complexity of in silico pathogenicity prediction.

Roger Colobran[1,2,3]; Elena Álvarez de la Campa[4]; Pere Soler-Palacín[2,5]; Andrea Martín-Nalda[2,5]; Xavier De la Cruz[4,6]; Ricardo Pujol-Borrell[1,2,3]; Mónica Martínez-Gallo[1,2,3]

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Immune dysregulation, polyendocrinopathy, enteropathy, X-linked (IPEX) syndrome is a rare monogenic primary immunodeficiency (PID), characterized by multi-organ autoimmunity including severe diarrhea due to enteropathy, chronic dermatitis, endocrinopathy (e.g. T1D, hypothyroidism) and other organ-specific diseases such as anaemia, thrombocytopenia, hepatitis and nephritis. It is caused by mutations in the transcription factor *FOXP3*, the master gene of T regulatory cells. Here we present a family with 5 siblings (3 boys and 2 girls) from no consanguineous parents; maternal uncle died at the age of 3 months because of hepatitis and diarrhea. Case 1: boy died at the age of 2 months because of infectious meningoencephalitis. Case 2: boy died at 13 months of age due to *Klebsiella* spp. sepsis, persistent diarrhea and severe skin lesions. Case 3: boy with several bacterial and fungal sepsis leading to death, severe eczema and diarrhea. DNA from the necropsy of case 2 was studied and the molecular analysis revealed a novel mutation in hemizygosity in the *FOPX3* gene (p.Phe367Val). Both the mother and the older sister were carriers of the same mutation. Since the mutation was new, we used several bioinformatic tools (i.e. Polyphen2, SIFT) to predict the pathogenocity of this mutation, but they gave us discordant results (benign vs damaging). Interestingly a similar discordance happened with other already described mutations affecting the same position of FOXP3 protein (p.Phe367Leu and p.Phe367Cys). We carried out an exhaustive bioinformatic analysis of these three mutations affecting the amino acid 367 and we compared our results with the discordant results obtained by the currently available *in silico* tools.

9 Aplicación clínica del cribado genotípico del C4 para el seguimiento personalizado de pacientes con LES

Rebeca Sánchez-Hidalgo[1]; Roger Colobran Oriol[1]; Cristina Sole Marce[2]; Josefina Cortes Hernandez[2]; Eduard Muñiz Diez[3]; Josep Ordi Ros[2]; Ricardo Pujol-Borrell[1]; Manuel Hernández Gonzalez[1].

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Los pacientes con lupus eritematoso sistémico (LES) presentan un curso clínico caracterizado por episodios de actividad y remisión. Para valorar la actividad de la enfermedad, a los pacientes se les suele monitorizar midiendo los anticuerpos anti-ADN nativo, el C3, y el C4. Existe un grupo de pacientes con LES que mantienen una concentración baja de C4 en suero, a pesar de responder bien al tratamiento, lo que dificulta una decisión en las pautas terapéuticas. La genética de C4 es compleja ya que está codificado por los genes C4A y C4B y éstos pueden estar en un número variable de copias (CNV). C4A y C4B solamente se diferencian en cuatro aminoácidos que les confiere una diferente funcionalidad, el C4A tiene un papel más importante en la eliminación de inmunocomplejos, y C4B en la lisis de las bacterias patógenas. El objetivo de este trabajo es averiguar: 1) si la no restauración de los niveles normales de C4 en pacientes con lupus en un estado inactivo de la enfermedad tras tratamiento podría ser explicada por la presencia de un defecto primario de C4 y 2) si el defecto total de C4A (C4Q0) en los pacientes está asociado a una mayor tasa de inmunocomplejos en suero y 3) el defecto total de C4B (C4BQ0) a una capacidad hemolítica disminuida.
Material y métodos: 134 donantes sanos. 55 pacientes con LES, grupo 1: n=29 tras tratamiento no restauraban los niveles normales de C4 y grupo 2: n=26restauraban los niveles normales al recibir tratamiento. 1) Se realizó un cribado genotípico de C4 por RFLP con la enzima de restricción BspLI que permitió diferenciar C4AQ0 de C4BQ0 y del genotipo silvestre (C4A/C4B). La confirmación fenotípica se realizó mediante la prueba serológica Chido/Rodgers (Dr. Muñiz, BST). 2) Los estudios funcionales *in vitro* de las proteínas C4A y C4B se hicieron con suero deplecionado de C4 a los que se añadió suero de donantes C4QA y C4BQ0, los parámetros a valorar fueron el CH50 (Wako) y la formación de C5b9 con estímulos de la clásica y de la MBL (Wielisa). 3) La concentración de C4d y C5b9 en suero se utilizó para valorar el de estado activación del complemento en los pacientes (ELISA comercial de MicroVue™). 4) La concentración Inmunocomplejos circulantes (ICC) en suero con un método basado en la unión a C1q (ELISA comercial de MicroVue™).
Resultados: 1) RFLP: sanos C4AQ0 (1/134) y C4BQ0 (5/134). Grupo 1: C4AQ0 (1/29), C4BQ0 (0/29) Grupo 2: C4AQ0 (0/26), C4BQ0 (0/26), los genotipos fueron confirmados con la prueba serológica Chido/Rodgers. 2) Estudios *in vitro* funcionales de C4A y C4B: La capacidad hemolítica del C4B fue 5 veces superior a la de C4A tanto para el CH50 como para el Wielisa. 3) Estado activación del complemento en los pacientes: se encontró una diferencia significativa ($p<0,05$) entre la concentración de C4d entre el grupo de donantes sanos (7 ± 3,1mg/mL) y los grupos con lupus: grupo 1 (12,6 ± 2,5 mg/mL) y grupo 2 (14 ± 2,5mg/mL). Aunque la casuística es muy baja y no se pueden hacer estudios estadísticos se ve una cierta asociación entre la concentración de C4d y la concentración de C4 total. Los donantes sanos con alelos nulos (C4AQ0: 3,5mg/mL/ C4BQ0: 5,3 ± 1,1mg/mL) tienen menor concentración de C4d que los sanos con C4A y C4B (7,8 ± 2,5 mg/mL). 4) ICC: Se encontró una diferencia significativa ($p<0,05$) entre el grupo donantes (3,03 ± 0,9 mg Eq/mL) y el grupo 1 (7,04 ± 1,7 mg Eq/mL). Hubo diferencia significativa ($p<0,05$) entre el grupo 1 y el 2 (4,33 ± 0,85mg Eq/mL). Aunque la casuística es muy baja y no se pueden hacer estudios estadísticos se ve una cierta asociación positiva entre la concentración de ICC y el genotipo C4AQ0: 4,7 mg Eq/mL (VR >4 mg Eq/mL).
Conclusiones: Este trabajo proporciona unos resultados preliminares que deberán ser confirmados con el estudio de más pacientes con LES y donantes, que sirven para proponer un algoritmo de seguimiento de los pacientes con LES en donde además de las pruebas recomendadas internacionalment se haga un cribado genotípico (RFLP del C4), posteriormente una cuantificación del número de copias de C4A y C4B y después una cuantificación de la concentración de C4d, que en función del CNV podremos estimar si la concentración de C4 es debida al consumo o al genotipo.

10 Diagnostic of Familial Hemophagocytic Lymphohistiocytosis type 5 (FHL5) caused by mutations in STXBP2 gene

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Familial Hemophagocytic Lymphohistiocytosis type 5 (FHL5) is caused by genetic mutations in *STXBP2* gene, which encodes for the protein Munc18-2. Munc18-2 interacts with the protein Syntaxin11 for thelytic granule exocytosis playing a critical role in NK and Cytotoxic T Lymphocytes mediated cytotoxicity.

We report the case of 2 years-old boy from non-consanguineous parents of Russian origin, who developed recurrent EBV-HLH in November 2013 and February 2014. Family history was positive for brother who died from EBV-HLH at the age of 3 years-old. The functional *in vitro* assays showed an impaired cytotoxic activity, which was almost absent without active HLH. The expression of intracellular perforin was normal. The percent of responding NK cells expressing CD107a after stimulation was similar to a healthy control. However, the analysis of the CD107a Mean Fluorescence Intensity (MFI) index of CD107a was significantly reduced when compared with healthy controls, which was instrumental in confirming the degranulation defect.

The genetic analysis identified a compound heterozygous mutation in *STXBP2* gene. A novel mutation c.728T>G located at exon 9 led to the aminoacid change Leu243Arg. The *in silico* analysis with the bioinformatics programs Polyphen2 and SIFT suggest that this amino acid is predicted to be critical for protein integrity. The mutation c.1247-1G>C affects the splicing site of exon 15, leading to a non-functional protein, which has already been reported. Both parents were carriers of the mutations. At the time of the diagnosis, the mother got pregnant and preconception council could be carried out.

Patients with FHL5 may be not detected by degranulation assays if only the percent of responding NK cells expressing CD107a is taken into account. Herein we highlight the importance of the MFI index for the diagnostic of FHL5, as a complementary data of degranulation assays.

11 Mucosal Immunity in fish: learning from lower vertebrates.

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Nonclassical animal models (invertebrate and vertebrate) have been useful for delineating the evolutionary history of immune reactions and can provide a basis for the discovery of previously unknown molecules and biochemical pathways involved in mammalian immunity. Excellent examples of this are the seminal discoveries by Jules Hoffmann of the Toll and Imd defense molecules and pathways in *Drosophila*, for which he was awarded the 2011 Nobel Prize in physiology or medicine. Such studies provided essential insights useful for the later discovery of Toll-like receptors in mammalian systems. Teleost fish have also contributes to several newly discoveries in mammalian immunology, such as phagocytic B cells or IgD+ B cell population. Fish present extensive mucosal surfaces: gut, skin and gills, and all of them contain mucosa-associated lymphoid tissue that serves a pivotal role in the maintenance of mucosal homeostasi. Mucosal immunoglobulins and B cells in teleost fish constitute the most ancient mucosal immunoglobulin-based system thus far described. Thus, IgT has been described as the first immunoglobulin in evolution specialized in mucosal responses. Sharing many characteristics of mammalian IgA, IgT function has been characterized in gut, skin and gills, playing an important role in the immune responses in all three tissues. Interstengly, fish lack germinal centers or lymph nodes, generating all mucosal immune responses in the extrafollicular compartment. Additional discoveries about fish mucosal immunity are likely to spark new knowledge applicable to the understanding of unresolved paradigms of mammalian mucosal immunity.

12 Gut Microbial Antigen Specific CD4$^+$T cells from Crohn's Disease Patients exhibit a Pro-inflammatory Th17 Phenotype

Elisabeth Calderón-Gómez[1]; Helena Bassolas-Molina[1]; Rut Mora-Buch[1]; Isabella Dotti[1]; Núria Planell[4]; Raquel Cabezón[5]; Sharat Singh[3]; Julian Panés[1,2]; Daniel Benítez-Ribas[4]; Azucena Salas[1].

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Experimental models have led to the theory that chronic inflammation, as seen in Crohn's disease (CD), results from a loss of tolerance towards commensal microbiota. In fact, antibodies specific for microbial components are found in 50% of CD patients, indicating that a memory T-cell response might be generated as well. There is yet little evidence, however, in human disease proving the later. In testing reactivity to several gut microbial antigens in peripheral blood, we have detected T-cell responses towards antigens including FlaX, Fla2 and YidX in both healthy individuals and CD patients. Interestingly, the proliferative response was significantly higher among CD patients compared to controls, which also correlated with an increased production of IFN-γ and IL-17 as assessed by ELISA. Intracellular cytokine staining showed the presence of Th17, Th1 and Th17/Th1 cells among the FlaX, Fla2 and YidX-specific T-cell populations, whose frequency was higher in CD. Furthermore, real time-PCR analysis of RORc, IL-17A and IL-17F expression on sorted FlaX, Fla2 and YidX-specific CD4$^+$T cells revealed a clear bias towards a pathogenic Th17 phenotype only in CD patients' T cells, but not in T cells from healthy controls. Thus, our data indicate that T cells that react to the same gut microbial antigen have been differently imprinted with a pro-inflammatory phenotype during CD. We hypothesize that these circulating memory T cells may contribute to sustain gut inflammation in CD upon antigen re-encounter; their identification opens new avenues for antigen-directed therapies in CD.

13 Efecte modulador de la deficiència de CD6 davant l'estimulació al·logènica

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CD6 és un receptor limfocitari físicament associat al complex de reconeixement específic de cèl·lules T (TcR) i B (BcR), que disposa d'una regió citoplasmàtica amb capacitat transductora de senyals intracel·lulars. Malgrat és capaç de modular les senyals desencadenades pel reconeixement d'antígens per part del TcR o BcR, la seva funció no està totalment caracteritzada. Amb aquest context es va voler estudiar l'efecte de la deficiència de CD6 sobre la resposta limfocitària a l'estimulació al·logènica tant *in vitro* com *in vivo*. D'aquesta manera, es va analitzarun model experimental de malaltia d'empelt contra hoste (o Graft versus Host Disease, GvHD) per transferència d'esplenòcits de ratolins B6.C-H-2^{bm12}/KhEg (bm12) a ratolins C56Bl/6 (B6) wild-type (WT) i a ratolins que tenien el gen de CD6 del·lecionat (CD6KO). Els sèrums analitzats procedents de sang perifèrica mostren un increment d'autoanticossos anti-DNAds i un augment del percentatge d'esplenòcits T $CD4^+$ $CD25^+$ $FoxP3^+$ i $CD8^+$ $CD69^+$ en ratolins CD6KO. Paral·lelament, es va fer una aproximació *in vitro* per mitjà de co-cultius cel lulars (*mixed lymphocyte reaction o MLR*), en els quals esplenòcits prèviament irradiats de ratolins bm12 es van posar en cultiu amb limfòcits T $CD4^+$ de ratolins CD6KO o de ratolins CD6WT. Els resultats obtinguts demostren un augment de l'activació i inducció de cèl·lules T reguladores, així com un major consum de citocines de tipus Th1 per part de cèl·lules CD6KO. En conclusió, les nostres dades son compatibles amb una funció de CD6 com a regulador negatiu de l'activació limfocitària davant d'un estímul al·logènic.

14 A founder splicing mutation in CD3δ in a Tαβ$^-$Tγδ$^+$B$^+$NK$^+$ SCID pedigree of ecuadorian descent

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Introduction: Severe Combined Immunodeficiency (SCID) is a group of rare and fatal-inherited disorders characterized by early onset of infections and diminished T cell number and impaired T cell function.

Aims: To identify the genetic and molecular alteration in an Ecuadorian patient with T$^{\pm}$B$^+$NK$^+$ SCID.

Methods: The patient, a 6-month-old girl of non consanguineous parents, presented with fever, eczema, and respiratory distress. CMV pneumonia was diagnosed, and active CMV infec chorioretinitis. The patient also presented regional lymphadenitis following BCG vaccination (BCGitis). Lymphopenia, and severe hypogammaglobulinemia were observed. Lymphocytes were determined by flow cytometry. CD3 gamma and delta genes were analyzed in DNA from patient and parents's peripheral blood leukocytes. Haplotypes, covering the CD3 region, were extensively analyzed.

Results: The patient showed a severe selective reduction in peripheral blood αβ T lymphocyte numbers (both CD4$^+$ and CD8$^+$). In contrast, γδ T cells, as well as B and NK lymphocytes, appeared in normal numbers (Tαβ$^-$Tγδ$^+$B$^+$NK$^+$ phenotype). TCR and CD3 expression levels were strongly impaired. Genomic DNA sequencing detected a homozygous G-to-A mutation at position splice donor site of intron 2 (IVS2$^+$5G>A). The patients' parents were carriers of the same CD3D mutation. This mutation has been previously described in two non-related families of Ecuadorian descent. The 3 families reported to date are unrelated, but had a similar geographic origin (Manabí region), indicating that they likely shared a frequent founder mutation, since we were able to identify several haplotypes involved.

Conclusions: We report a T$^{\pm}$B$^+$NK$^+$ SCID patient belonging to a complex pedigree with a homozygous splicing mutation in the CD3 delta gene (c.274$^+$5G>A).

15 Molecular and phenotypical analysis of transgenic mice for soluble human CD6 expression*

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CD6 is a membrane glycoprotein receptor expressed in thymocytes, mature T cells, B1a cells, NK cells, hematopoietic precursors and also in some brain regions. This molecule binds to CD166/ALCAM, an adhesion molecule mainly expressed in antigen presenting cells and thymic epithelial cells. CD6 possesses a long cytoplasmic tail suitable for signal transduction which is physically associated to the clonotypic receptor of T and B1a cells, so that it is well positioned to modulate initial steps of TCR and BCR signaling. However, the function of CD6 is yet to be fully described. Initially considered as a co-stimulatory molecule, a recent report proposes that CD6 may act as a negative modulator of TCR-mediated signalling. To further elucidate the putative role of CD6 in lymphocyte activation and development, we developed a transgenic C57Bl/6 mouse line (shCD6LckEu Tg) expressing a circulating soluble form of human CD6 (shCD6) under the transcriptional control of both the lymphocyte-specific promoter of the Lck tyrosine kinase and the Ig heavy chain enhancer (Eu), so this molecule will be preferentially expressed in T and B cells. We expected that shCD6 would work as a decoy receptor specifically blocking the ligand-receptor interactions mediated by membrane-bound CD6 and its ligand, resulting in a functional knocking-down of this protein.

The transgenic mice effectively expressed shCD6 in the serum at the ng/ml range and preliminary phenotypical analysis showed significant changes in some specific lymphocyte subpopulations both in thymus (decrease in the percentage of DN1 ($CD44^+CD25^-$) and CD8SP ($CD4^-CD8^+$) cells and an increase in the percentage of DN4 ($CD44^-CD25^-$)cells) and spleen (decrease in the percentage of Treg cells ($CD4^+CD25^+Foxp3^+$) and an increase in the percentage of NK cells ($CD3^-NK1.1^+$). Moreover the transgenic mice exhibit an increased anti-tumoral response in an autologous B16 melanoma model, a fact that is also observed following repeated infusion of wild-type mice with purified rshCD6.

The results obtained not only support a relevant role of CD6-mediated interactions in lymphocyte development as well as in homeostasis of some peripheral lymphocyte subsets, but also provides a new experimental model to explore the relevance of those interactions in different immune-related disorders.

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e-poster List

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The authors will attend the poster on **20/11/2014**: 17:30h; **21/11/2014**: 11:00h, 13:30h, 16:55h.

1 Intestinal gene expression in a model of food allergy in brown norway rats

Mar Abril-Gil[1,2]; Malén Massot-Cladera[1,2]; Francisco J. Pérez-Cano[1,2]; Àngels Franch[1,2]; Margarida Castell[1,2].

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Over the past years, food allergy (FA) has become a major public health problem in industrialized countries [1]. The development of a suitable and reproducible FA experimental model useful for the screening of new anti-allergic therapies as well as to understand the underlying mechanism of the disease has become a priority.

The aim of the present study was to characterize the changes in intestinal gene expression induced by a FA model in Brown Norway rats. Three-week old female rats were immunized by means of an intraperitoneal (i.p.) injection of an emulsion containing ovalbumin (OVA), Alum as an adjuvant, and toxin from *Bordetella pertussis* to promote IgE synthesis [2]. Fourteen days later, rats were administered orally with OVA for a week to induce FA. As a control, a reference group (RF) received vehicle by oral gavage. Blood samples were weekly collected to determine specific anti-OVA IgE and IgG1 antibodies. After an oral challenge with a high dose of allergen, body temperature, intestinal permeability, hematocrit and serum mast cell protease (RMCP)-II were determined to assess anaphylactic shock. At the end of the study, small intestine was collected to measure the gene expression by real-time PCR.

Serum concentration of specific anti-OVA IgE and IgG1 antibodies in FA group were already detectable at one week after i.p. immunization, and highly increased after the oral administration, confirming the achievement of a FA development. The anaphylactic shock induction produced hypothermia in FA group ($p < 0.05$) and also increased the hematocrit ($p < 0.05$) and the intestinal permeability with respect to RF group. Serum RMCP-II concentration in FA group rose an average of 28 times in comparison to RF animals ($p < 0.05$). Concerning intestinal gene expression, a tendency to increase RMCP-II as well as occludin could be measured. However, IgA, FcεRI, TGF-β and mucin gene expression tended to be down-regulated in FA group.

In conclusion, because of significant changes in the intestinal gene expression could not been detected, further studies involving a higher number of animals must confirm the current tendencies observed.

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[2] Dong W. Systemic Administration of Bordetella pertussis Enhances Pulmonary Sensitization to House Dust Mite in Juvenile Rats. Toxicol Sci 2003; 72: 113–21.

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The authors will attend the poster on **20/11/2014**: 17:30h; **21/11/2014**: 11:00h, 13:30h, 16:55h.

2 Modulation of the suckling rat rotavirus infection by a preventive hyperimmune bovine colostrum administration

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INTRODUCTION: Rotaviruses (RV) predominantly infect young children worldwide with a spectrum of disease, ranging from asymptomatic shedding, diarrhoeal disease, to severe dehydration and even death. Several animal models have been used to explore RV infection and its pathology, clinical and immune response, and to test vaccine efficacy or nutritional modulation of the process. As after RV infection, immunity is not complete and less severe re-infections usually occur; a multiple infection model would be necessary in order to study how preventive interventions can modulate such responses.
OBJECTIVE: The particular purpose of this study was to evaluate the influence of an effective modulator of the RV infection and its impact on the immune response developed during a second infection.
METHODS: For that, Lewis neonatal rats were inoculated with SA11 (first RV infection) on 6th day of life and with EDIM (second RV infection) on 17th day of life. Some of them were also orally administered with an anti-RV hyper immune bovine colostrum (HBC) between 3rd and 9th day of life. Clinical parameters such as diarrhoea incidence and severity, faecal pH, body temperature and immune response (rotavirus-specific antibody titters, ex vivo IFNγ production and rotavirus-specific DTH) were evaluated.
RESULTS: Besides a clinical controlling effect of HBC during first infection -by means of reduction in incidence, severity and duration of the process- the intervention allows rat immunization for future re-infections. Therefore, the capacity of the HBC treated animals to control a re-infection is similar or even higher as is shown by an increase in systemic and mucosal specific immune response.
CONCLUSION: The intervention with HBC prevents RV first infection and allows the protective immunity development against re-infection, suggesting the suitability of this double infection model as an experimental tool to study modulation of (re)infection by preventive interventions

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The authors will attend the poster on **20/11/2014**: 17:30h; **21/11/2014**: 11:00h, 13:30h, 16:55h.

3 Evidències de l'associació entre microbiota intestinal i T1D en el model murí 116C-NOD i NOD

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La diabetis mellitus tipus 1 (T1D) és una malaltia complexa que es caracteritza per la destrucció selectiva de les cèl·lules β pancreàtiques productores d'insulina. El sistema immune intestinal posseeix una funció clau en el curs de la T1D. Així mateix, la microbiota intestinal, com a factor regulador d'aquest sistema, també pren rellevància en l'autoimmunitat β pancreàtica. En aquest sentit, estudis en models animals mostren com el desenvolupament de la T1D és alterat per canvis en la microbiota intestinal induïts per antibiòtics i probiòtics.

El nostre grup ha generat recentment el model 116C-NOD, un ratolí transgènic portador dels gens de la cadena pesada i lleugera de la immunoglobulina de l'hibridoma H116, productor de l'anticòs AcMoH116 amb especificitat anticèl·lula β. El ratolí 116C-NOD presenta una incidència menor de diabetis en ambdós gèneres respecte el ratolí NOD, el model espontani de T1D.

L'objectiu del present treball és estudiar la relació entre la microbiota intestinal i la T1D mitjançant els models 116C-NOD i NOD. Per tal d'això, 1) s'estan avaluant el debut i la incidència de T1D en els ratolins 116C-NOD, així com en els seus germans NOD no transgènics mantinguts en convivència i en aïllament vers els primers, per a permetre o impedir la compartició de microbiota intestinal, respectivament; 2) s'està cercant reactivitat creuada dels limfòcits B del ratolí 116C-NOD amb antígens de microbiota intestinal, via anàlisi immunoproteòmica.

Els experiments d'estabulació evidencien que la convivència dels ratolins 116C-NOD amb els seus germans NOD disminueix la incidència i redueix la taxa d'aparició de T1D dels segons, en comparació amb ratolins NOD en situació d'aïllament vers els seus germans transgènics. Per altra banda, els estudis preliminars d'immunoproteòmica mostren que AcMoH116, i per tant, els limfòcits B del 116C-NOD reaccionen de forma creuada amb la proteïna EF-Tu 1 d'*Escherichia coli.* Els primers resultats suggereixen l'existència de poblacions de microbiota tolerogèniques o protectores, característiques del model 116C-NOD, que frenarien o retardarien la T1D en els ratolins NOD. Així mateix, la reactivitat creuada podria ser un dels mecanismes d'interacció entre els limfòcits B i la microbiota en la resposta autoimmunitària.

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The authors will attend the poster on **20/11/2014**: 17:30h; **21/11/2014**: 11:00h, 13:30h, 16:55h.

4 Expression of colonic genes related to immune function in wistar rats fed a cocoa-enriched diet

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Introduction: There is evidence that cocoadiet influences intestinal adaptive immune response by changing, among others, the proportion and functionality of T and B cells located in the gut-associated lymphoid tissue (GALT)[1,2]. However, the mechanisms and pathways involved remain unidentified.

Objective: The aim of the present study was to deep into the immunomodulatory effects of a cocoa diet by evaluating its impact on the expression of genes related to immune function in the intestinal compartment.

Methods: Three-week-old female Wistar rats were fed either a standard diet or a diet containing 10% cocoa with 0.4% polyphenols. After three weeks, colonic RNA was obtained to evaluate the differential gene expression using the SurePrint-G3 Rat Gene Expression Microarray (Agilent). Changes found in the expression from highly modified genes are currently being validated by Real Time-PCR.

Results: A 10% cocoa diet for three weeks regulated a great number of genes involved in immune response-related pathways. To date, genes involved in T cell proliferation (GO:0042130), immunoglobulin (GO:0002377) and cytokine production (GO:0001816), and mast cell functionality (GO:0045576; GO:0043303) were down-regulated. At the same time, cocoa up-regulated the lymphocyte activation (GO:0051249) and migration (GO:2000403) pathways.

Conclusion: Cocoa intake affects intestinal gene expression of several pathways involved in the intestinal immune response which may explain the immunomodulatory action ascribed to cocoa.

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5 Epithelial IL-1R2 acts as a homeostatic regulator of inflammatory signals during remission of ulcerative colitis

Rut Mora-Buch[1]; Isabella Dotti[1]; Nuria Planell[2]; Elisabeth Calderón-Gómez[1]; Azucena Salas[1].

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Ulcerative colitis (UC) is an intestinal inflammatory disease that evolves with active flare-ups followed, in most patients, by therapy-induced remission. We focused on identifying intrinsic regulatory mechanisms that favor remaining long-term mucosal homeostasis during disease remission. A total of 180 samples from UC patients and 55 from healthy non-IBD controls participated in the study. Using real-time PCR and ELISA of culture supernatants from colon biopsies, we found that the interleukin-1 (IL-1) decoy receptor gene (*IL1R2*) and secreted protein (IL-1sR2) were up-regulated during UC in remission compared to active UC and non-IBD controls. In contrast, the IL-1 receptor antagonist (IL-1Ra) together with IL-1β, IL-1 receptor type 1 (IL-1R1) and IL-1 receptor accessory protein (IL-1RAcP) were overexpressed in active UC samples. By immunostaining we identified both lamina propria IgA^+ cells and adjacent mucosal epithelium as positive for IL-1R2.Nevertheless, by intracellular flow cytometry staining of digested biopsies, we found that epithelial cells increased intracellular IL-1R2 production during UC in remission. Culturing whole colonic crypts or epithelial stem cell, we observed that IL-1R2 expression was diminished by Wnt/beta-catenin signals, thus epithelial stem cells up-regulate IL-1R2 upon differentiation. Importantly, blocking IL-1R2 in isolated colonic crypts from biopsies of UC patients in remission boosted IL-1β-dependent secretion of CCL20. Furthermore, expression of the *IL1R2* in the remitting mucosa could be a predictive molecule of UC relapse during a 1-year follow-up. In summary, in contrast to IL-1Ra, increased epithelial IL-1R2 expression may represent an endogenous homeostatic mechanism during remission, which could restrain the trigger of the inflammatory response.

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The authors will attend the poster on **20/11/2014**: 17:30h; **21/11/2014**: 11:00h, 13:30h, 16:55h.

6 Butyrate induces damaged intestinal mucosa repair and stimulates IL-1b secretion.

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IL-1b is commonly considered a pro-inflammatory cytokine produced in several pathological conditions, including colitis. However, the role of IL-1b production during intestinal inflammation is still controversial. Secretion of IL-1b is mediated through the activation of inflammasomes. Although some studies support an inflammatory role of inflammasomes in the intestinal mucosa, the majority of studies have shown a protective role of inflammasome activity in controlling intestinal inflammation and homeostasis. Butyrate is a short-chain fatty acid produced during colonic fermentation that also plays a pivotal role in the maintenance of intestinal homeostasis. Moreover, it has been demonstrated that butyrate enemas or dietary intake of fiber ameliorates DSS induced colitis in mice.

Using bone marrow derived macrophages, colonic explants and isolated intestinal macrophages from wild-type and NLRC4 KO mice, we have demonstrated that butyrate induces the secretion of IL-1b in BMDM and intestinal samples through a NLRC4-dependent inflammasome. Moreover, IL-1b or conditioned medium obtained from butyrate-treated macrophages stimulate the repair of a physically damaged monolayer of intestinal epithelial CMT-93 cells. Accordingly, in vivo studies suggest, that butyrate induces the early secretion of intestinal IL-1b in DSS-colitic animals and diminishes inflammation, representing two effects induced by butyrate, which are abrogated in DSS-colitic NLRC4 KO mice.

Therefore, our results suggest that butyrate is acting as a DAMP (damage-activated molecular pattern) capable to activate the NLRC4 inflammasome and induce the secretion of intestinal IL-1b, which seems to be involved in the repair of the damaged mucosa.

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The authors will attend the poster on **20/11/2014**: 17:30h; **21/11/2014**: 11:00h, 13:30h, 16:55h.

7 Immunological studies in paediatric patients with Immflamatory Bowel Disease (IBD)

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Intestinal disease in children makes up a diverse group of diseases including genetic, structural and acquired diseases.It has been described that a proportion of patients with early onset IBD suffer from monogenic disorders. Some of these monogenic disorders are Primary Immunodeficiency Diseases (PID). Our objective is to screen paediatric patients with enteropathy to discard potential PID that could be the underlying cause of the intestinal manifestations.

Patient selection was done according to the diagnostic criteria established by the IBD working-group of the ESPGHAN. Four groups were defined attending to different features of the disease: early onset, bad evolution, extraintestinal manifestations, and food intolerance. Lymphocyte subpopulations T, B and NK, and HLA-DR, TCRαβ/TCRγδ, and $CD45RA^+/CD45RO^+$ expression was analysed by flow cytometry. Oxidative burst assay was done. *In vitro* production by PBMCs of TNF-α after LPS and IL-10 stimulation was done in order to explore interleukin (IL)-10 signalling defects.

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The authors will attend the poster on **20/11/2014**: 17:30h; **21/11/2014**: 11:00h, 13:30h, 16:55h.

8 Changes in intestinal gene expression induced by oral sensitization with ovalbumin in lewis rats

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In healthy conditions, oral tolerance is produced in response to antigens encountered via the gastrointestinal tract. However, when oral tolerance is broken, allergy to food components appears [1]. Animal models of food allergy can provide advances in understanding the pathogenesis as well as developing therapeutic strategies. Rodent models of food allergy induced by oral sensitization suppose the breakdown of oral tolerance mechanisms. In this study, we aimed to characterize the gene expression of some molecules involved in both the intestinal immune response and oral tolerance in rats orally sensitized with ovalbumin (OVA).

Three-week-old female Lewis rats received, by oral route, three times per week and for three weeks, a solution of OVA together with choleric toxin as an adjuvant capable of breaking oral tolerance [2]. Serum anti-OVA IgG1, IgG2a, IgG2b, IgG2c, IgE, IgA and IgM were quantified by ELISA. At the end of the study, a middle piece from the small intestine was collected to measure the gene expression of IgA, Foxp-3, TGF-b, IL-4, IL-6, IL-10, IL-17a, TLR-4, TLR-5, claudin, occludin and mucin by real-time PCR.

All sensitized rats developed anti-OVA IgG antibodies, mainly belonging to the IgG2a isotype, which is related to Th2-immune response in rats. Although a certain specific IgM and IgA synthesis was also produced, no specific IgE could be detected. With respect to changes in the intestinal tissue, no significant mRNA levels of claudin, IL-6, IL-17α and IL-4 were expressed in the small intestine wall which could be due to the fact that mRNA was obtained from the entire intestine wall and not from intestinal lymphocytes. Neither IgA, occludin nor mucin gene expressions varied significantly in orally sensitized animals suggesting no defects in the gastrointestinal barrier. TLR-4 showed little change, but TLR-5 underwent a down-regulation in sensitized rats which agrees with the role of TLR-5 in the prevention of murine intestinal allergy [3]. IL-10 gene expression was partially down-regulated in orally sensitized animals, suggesting the breakdown of oral tolerance. However, TGF-β, another tolerogenic cytokine, did not change and neither did Foxp3, suggesting that the number of Treg could be kept even in these conditions.

In conclusion, although some changes were detected in orally sensitized rats, the isolation of lymphocytes from the intestinal lamina propria could allow obtaining more significant results. This study was supported by a grant from the Ministerio de Economía y Competitividad (AGL2011-24279).

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The authors will attend the poster on **20/11/2014**: 17:30h; **21/11/2014**: 11:00h, 13:30h, 16:55h.

9 Caracterización de la respuesta inmune en pacientes con EPOC

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La EPOC se asocia a una respuesta inflamatoria pulmonar y sistémica anormal a gases nocivos, en nuestro entorno principalmente el humo del tabaco. El objetivo de este trabajo es caracterizar y relacionar la respuesta inmune celular a nivel pulmonar y sistémico en pacientes con EPOC, y compararla con la que se da en controles fumadores con función pulmonar normal y controles no fumadores.

Hasta la fecha se han estudiado muestras de tejido pulmonar fresco y sangre periférica obtenidas en 22 pacientes con EPOC, 7 fumadores con función pulmonar normal y 9 individuos no fumadores que precisaron cirugía de resección pulmonar por motivos clínicos (generalmente presencia de tumor pulmonar). Se ha utilizado citometría de flujo para determinar en estas muestras las poblaciones de linfocitos T ($CD4^+$ y $CD8^+$) y B, neutrófilos, macrófagos, monocitos, células dendríticas y células NK y NKT.

En tejido pulmonar se observa un mayor número de macrófagos y linfocitos B en pacientes con EPOC vs. controles. Además, los macrófagos presentan una mayor proporción de fenotipo mixto con expresión de marcadores pro-inflamatorios ($CD80^+$) y anti-inflamatorios ($CD163^+$). Finalmente, es destacable que estas diferencias observadas en tejido pulmonar no se observan en sangre periférica.

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The authors will attend the poster on **20/11/2014**: 17:30h; **21/11/2014**: 11:00h, 13:30h, 16:55h.

10 RORc antagonists inhibit il-17 production by gut commensal-specific T cells in Crohn's disease

Helena Bassolas-Molina[1]; Núria Planell[3]; Míriam Esteller[3]; Ernest Raymond[4]; Gerald Nabozny[4]; Julià Panés[1,2]; Azucena Salas[1]; Elisabeth Calderón-Gómez[1]

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Background: Crohn's disease (CD) is a chronic inflammatory disorder of the gastrointestinal tract. Increasing evidence suggests that disease results from an aberrant immunological reaction to a subset of commensal microorganisms. Since deregulated pathways include Th17 responses, the inhibition of RORc, the master transcriptional regulator of Th17 cells, represents a potential therapeutic strategy. Our aim was to compare the efficacy of two RORc inhibitors (Compound A and B) in a commensal-specific system that uses peripheral blood mononuclear cells (PBMCs) from CD patients and healthy individuals.

Methods: PBMCs were isolated and cultured with a panel of commensal antigens (ASCA, FliC, FrvX). Heat-killed Candida albicans was used as a positive control for IL-17 production and Tetanus Toxin as a non-commensal derived antigen. IL-17 production was measured by ELISA. Antigen-specific cytokine-producing cells were identified by flow cytometry using CFSE staining. The inhibitors' effect on other soluble cytokines was assessed by a cytokine multiplex assay. Next-generation sequencing was performed on PBMCs cultured with pooled antigens (ASCA, FliC, and FrvX) in the presence of compound A.

Results: We observed T cell-derived IL-17 production upon antigen stimulation in both CD and healthy individuals. IL-17 production was significantly higher in CD patients when stimulated with FrvX and pooled antigens compared to healthy individuals. Both RORc inhibitors reduced IL-17 secretion. However, compound A showed a more dramatic effect on IL-17 secretion than Compound B. Multiplex cytokine analysis confirmed a marked decrease in IL-17 production by both inhibitors. In contrast, neither of the two RORc antagonists impacted the production of any other cytokines tested, suggesting a clear target specificity for these compounds. Furthermore, Next-generation sequencing revealed a down-regulation of Th17 related genes such as IL-17A, IL-17F, IL-22, IL-26, and IL-23R, but did not alter the transcription of RORc.

Conclusions: Our findings provide evidence of the existence of gut commensal-specific RORc-driven Th17 cells in CD. Moreover, we demonstrate that RORc antagonists specifically block IL-17 secretion and transcription induced by microbial stimulation, while not interfering with production of other pro- or anti-inflammatory cytokines. Therefore, RORc antagonists could represent a highly specific therapeutic approach to CD treatment.

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The authors will attend the poster on **20/11/2014**: 17:30h; **21/11/2014**: 11:00h, 13:30h, 16:55h.

11 Immune-dependent antineoplastic effects of cisplatin plus pyridoxine in non-small-cell lung cancer

Fernando Aranda[1]; Norma Bloy[3]; Oliver Kepp[2]; Allan Sauvat[3]; David Enot[2]; Laurence Zitvogel[2]; Guido Kroemer[2]; Laura Senovilla[2]

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cis-Diamminedichloroplatinum(II) (CDDP), which is mostly referred to as cisplatin is a widely used antineoplastic. The efficacy of cisplatin can be improved by combining it with the vitamin B6 precursor pyridoxine. Here, we evaluated the putative synergistic interaction of CDDP with pyridoxine in the treatment of an orthotopic mouse model of non-small-cell lung cancer (NSCL). CDDP and pyridoxine exhibited hyperadditive therapeutic effects. However, this synergy was only observed in the context of an intact immune system and disappeared when the otherwise successful drug combination was applied to the same NSCLC cancer implanted in the lungs of athymic mice (wich lack T lymphocytes).

Immunocompetent mice that had been cured from NSCLC by the combined regimen of CDDP plus pyridoxine became resistant against subcutaneous rechallenge with the same (but not with an unrelated) cancer cell line. In vitro, CDDP and pyridoxine did not only cause synergistic killing of NSCLC cells but also elicited signs of immunogenic cell death including an endoplasmatic reticulum stress response and exposure of calreticulin at the surface of the NSCLC cells. NSCLS cells treated with CDDP plus pyridoxine in vitro elicited a protective anticancer immune response upon their injection into immunocompetent mice.

Altogether, these results suggest that the combined regimen of cisplatin plus pyridoxine mediates immune-dependent antineoplastic effects against NSCLC.

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The authors will attend the poster on **20/11/2014**: 17:30h; **21/11/2014**: 11:00h, 13:30h, 16:55h.

12 TSHR Stimulating Antibodies (TSAbs) from Graves' disease patients stimulate thymocytes and may contribute to the tuning/modulate of its own immune response

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Graves disease (GD) is a remarkable autoimmune disease defined by the production of stimulating autoantibodies to the TSHR (TSAbs) that induce a sustained state of hyperthyroidism. We have previously demonstrated that THSR is a key GD susceptibility gene, being the target of the immune response in these patients. We have also shown that this gene is unexpectedly expressed in thymocytes, however its role remained unsolved. On the other hand, a common feature of GD is the occurrence of a thymic hyperplasia, which could be explained by the action of TSAbs on TSHR-expressing thymocytes. More interestingly, constant thymocyte-TSHR activation by TSAbs could favor the escape of autoreactive T-cells from the thymus. Elucidating whether TSHR in thymocytes could be triggered by GD TSAbs is key to understand TSHR physiology and how it may contribute to the pathogenesis of GD. In this report, we confirm the expression of TSHR in thymocytes by Western blot and the presence of full-length TSHR mRNA by standard RT-PCR. We show that THSR expression in thymocytes is confined to the immature stages of development and decaying abruptly once the T-cells are released from the thymus, suggesting a role in thymocyte maturation. And finally, the bovine TSH, a human monoclonal antibody (M22) that mimics TSAbs, human sera and more importantly, purified IgGs with stimulating activity (TSAbs) all from GD patients, could significantly induce in a dose-response manner the production of intracellular cAMP levels in freshly isolated human thymocytes above basal levels. A monoclonal antibody with blocking activity to the TSHR (Ki-70) and a normal mouse serum did not induce activation of the TSHR. The cell line HEK-TSHR and primary human thyrocytes were used as reference control substrates. From these results, it is clear that THSR thymocytes can signal upon activation by TSAbs, which may contribute to explain the thymic hyperplasia observed in GD, and most interestingly, could contribute to the early stages of the autoimmune response to TSHR, facilitating the escape of autoreactive T-cells.

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The authors will attend the poster on **20/11/2014**: 17:30h; **21/11/2014**: 11:00h, 13:30h, 16:55h.

13 Adalimumab regulates intracellular TNF-α production in patients with rheumatoid arthritis

Carlos Zamora-Atenza[1]; Cesar Diaz-Torne[2]; Carme Geli[2]; Cesar Diaz-Lopez[2]; M. Angels Ortiz[1]; Patricia Moya[2]; Ivan Castellví[2]; Juan C. Nieto[1]; Elisabet Cantó[1]; Jordi Casademont[2]; Candido Juárez[2]; Josep M. Llobet[2]; Silvia Vidal[1]

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Introduction: Adalimumab is a fully human anti-TNFα monoclonal antibody that specifically blocks the interaction of TNFα with its receptors. It binds both soluble and transmembrane TNF-α. We hypothesized that blocking these TNF-α signals regulates the altered TNF-α production in rheumatoid arthritis (RA) patients.

Methods: We compared by flow cytometry TLR-induction levels of membrane and intracellular TNFα in monocytes (iTNFα$^+$CD14$^+$cells) from 12 patients before and after adalimumab treatment with those from 5 healthy donors.

Results: Before starting the treatment, the percentage of iTNF-α$^+$CD14$^+$ cells in patients was significantly lower than in healthy donors (33.16±4.82 vs66.51±2.4 %, p<0.001). When we added in vitro TNF-α to healthy donor culture cells, levels of iTNF-α CD14$^+$ cells decreased, suggesting that the TNF-α signal is responsible for the iTNFα$^+$CD14$^+$ cell downregulation observed in patients. After 2, 6 and 12 adalimumab injections, we observed significant blocking of membrane and soluble TNF-α and a progressive increase in iTNF-α$^+$CD14$^+$ cells in 10 patients with a good/moderate EULAR response. Levels of iTNF-α$^+$CD14$^+$ cells after 12 injections in these 10 patients were comparable to levels in healthy donors. In two patients, iTNF-α$^+$CD14$^+$ cell upregulation was not observed and EULAR response did not improve. The first patient developed anti-adalimumab antibodies, explaining why adalimumab was not able to block membrane and soluble TNF-α. In the second patient, adalimumab was discontinued due to adverse effects and iTNF-α$^+$CD14$^+$ cells then decreased to levels before treatment.

Conclusions: Our findings suggest that adalimumab treatment in RA patients can return intracellular TNF-α level to that of healthy donors. This effect was not observed in the presence of neutralizing anti-adalimumab antibodies.

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14 Evaluación de los niveles de anti-TNF-α e IL-6 en AR en correlación con la actitud terapéutica: Resultados preliminares

Bibiana Quirant Sánchez[1]; Melania Martínez-Morillo[2]; Anne Riveros[2]; Samantha Rodríguez-Muguruza[1]; Lourdes Mateo[2]; Susana Holgado[2]; Jerónima Cañellas[2]; Alejandro Olivé[2]; Xavier Tena[2]; Eva M. Martínez-Cáceres[1]; Aina Teniente-Serra[1]

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INTRODUCCIÓN: En los últimos años, las terapias biológicas, especialmente los inhibidores del factor de necrosis tumoral (anti-TNF-α), son parte fundamental en el tratamiento de la artritis reumatoide (AR). Los anti-TNF-α más usados son infliximab, adalimumab y etanercept. También se dispone de otras dianas terapéuticas como interleucina (IL)-6 (tocilizumab). Conocer los niveles del fármaco y los anticuerpos anti-fármaco podría ser un dato objetivo y complementario a la evaluación clínica, para conseguir optimizar el tratamiento de los pacientes.
OBJETIVOS: 1- Determinar niveles de fármacos así como la presencia de anticuerpos anti-fármaco en pacientes con AR tratados con anti-TNF-α (etanercept, adalimumab) y anti- IL6 (tocilizumab). 2- Evaluar si existe un cambio en la actitud terapéutica del reumatólogo tratante previa y posteriormente a la determinación de niveles de fármacos y anticuerpos.
MÉTODOS: Se incluyeron 40 pacientes (7 hombres/33 mujeres, media edad 49 años) diagnosticados de AR según los criterios del American College of Rheumatology (2010) en tratamiento con tocilizumab (n=12), etanercept (n=13) y adalimumab (n=15). En todos ellos ser recogieron los datos epidemiológicos, y el índice de actividad DAS-28. La determinación de los niveles de fármaco y anticuerpos anti-fármaco se realizó por ELISA (Theradiag) siguiendo las instrucciones del fabricante.
RESULTADOS: La media del DAS-28 de estos pacientes era de 2,9, y un 67,5% estaban en remisión o con baja actividad de la enfermedad (DAS-28 < 3,2). Se codificó la actitud terapéutica del reumatólogo según 7 posibilidades antes y después de conocer el resultado de los niveles de fármaco y anticuerpo anti-fármaco en los pacientes (tabla 1). Se cambió de actitud terapéutica en 19 pacientes (47,5%). En la mayoría de casos se disminuyó la dosis del fármaco o se modificó por otro (fracaso terapéutico). Únicamente uno de los pacientes presentó anticuerpos anti-fármaco (adalimumab). En dicho paciente se decidió interrumpir el tratamiento. En la tabla 1 se muestran los resultados. En relación al tocilizumab, debido a la heterogeneidad entre pacientes observada en los niveles de fármaco en suero y no estar bien definidos los niveles terapéuticos, se necesitan más estudios para establecer la relación entre la clínica y los niveles de fármaco.
CONCLUSIÓN: Los resultados de este estudio preliminar muestran que la determinación de los niveles de fármaco y los anticuerpos antifármaco pueden ser útiles en la toma de decisiones terapéuticas, y en la optimización del gasto de fármacos biológicos. Su aplicabilidad práctica podría basarse en la determinación de los niveles de fármaco, y únicamente si estos fueran bajos, se determinarían los anticuerpos anti-fármaco.

		Estrategia **posterior** a la determinación de niveles						
Estrategia **previa** a la determinación de niveles		Sin cambios	Aumentar la dosis	Disminuir la dosis	Suspender el tratamiento	Cambiar tratamiento biológico	Aumentar la dosis de inmunomodulador	Disminuir la dosis de inmunomodulador
	Sin cambios	**20 (50%)**		**13 (32,5%)**		**4 (10%)**		
	Aumentar la dosis					**1 (2,5%)**		
	Disminuir la dosis							
	Suspender el tratamiento				**1 (2,5%)**			
	Cambiar el tratamiento biológico							
	Aumentar la dosis de inmunomodulador					**1 (2,5%)**		
	Disminuir la dosis de inmunomodulador							

Tabla 1. Resultados de la actitud terapéutica según 7 posibilidades antes y después de conocer los niveles de fármaco y anticuerpo anti-fármac0.

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15 Preliminary evaluation of two IIF methods of anti-F-actin antibodies detection

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INTRODUCTION:
Autoimmune hepatitis type-1 (AIH-1) is characterized by the presence of antinuclear antibodies and/or anti-smooth muscle antibodies (ASMA) with or without F-actin specificity, according to international criteria. ASMA can be found in 85% of AIH-1 patients, although it is not specific of the disease. However, the presence of anti-actin has been described as being more specific for AIH-1. Detection methods for those antibodies range from various IIF techniques to ELISA and Immunoblot, but so far none of the antigen specific methods has given reliable results. The aim of this study is to compare the diagnostic strength of two IIF methods based on different substrates, rat triple tissue (Kidney-Stomach Liver, KSL) and rat intestine epithelial cells (RIEC).

METHODS:
We included 49 samples from evaluated patients with AIH (n=23), other hepatic diseases (n=4), and patients with other pathologies known to show ASMA (n=22). We used the kidney-stomach-liver (KSL) rat triple tissue sections and RIEC slides, developed with anti-Igs and anti-IgG fluorescent conjugates respectively (INOVA Diagnostics).

RESULTS:
We obtained 23 positive actin pattern with KSL and 24 with RIEC slides (kappa=47.38%, CI 20.67% - 74.09%). 7 samples gave an undefined pattern with the KSL slides, of which only 2 were weakly positive with the RIEC. Positive actin pattern was also detected in patients with other than AIH diagnosis with both methods (14/27 vs 14/27).

CONCLUSION:
Although both methods show similar numerical results, the visualization of the pattern in the rat intestine epithelial cells proved to be easier and simpler to identify when compared to the rat triple tissue.

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16 Anticossos anti-nucleosomes i lupus eritematós sistèmic: dades preliminars a l'Hospital Universitari de Bellvitge

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Objectiu

Valorar resultats preliminars de l'estudi en curs de la utilitat dels anticossos anti-nucleosoma (ANUC) en el diagnòstic i seguiment de pacients amb LES, en comparació als anticossos anti-DNA.

Material i mètodes

S'estudien retrospectivament tots els pacients amb determinacions simultànies d'anticossos antinuclears, anti nDNA i ANUC efectuades durant l'any 2013 i 2014 pel servei d'Immunologia del HUB. S'han recollit dades de 164 pacients atesos al HUB. Les mostres s'han processat per IFI (ANA), FEIA + IFI (nDNA) i dot-blot + ELISA (ANUC). En aquest estudi preliminar, s'han considerat només les mostres positives per anti-nucleosoma (blot) i/o anti nDNA del 2013.

Resultats

Del total de 164 pacients estudiats, s'han seleccionat 38 casos amb LES i positivitat per ANUC i/o nDNA. En 23/38 es detectaren anticossos anti-nDNA mentre que 34/38 mostraren reactivitat ANUC. Aquestes dades preliminars mostrarien una major sensibilitat dels ANUC respecte els anti-nDNA. Els altres pacients amb positivitat ANUC presentaren principalment: psoriasi, glomerulonefritis MPO+, VIH/VHC, SAF, connectivopaties, Crohn, HAI, pèmfig i AR, molts d'ells tractats amb fàrmacs biològics. L'especificitat dels ANUC per al LES va augmentar amb el títol (qualitatiu) d'autoanticossos, des d'un 41% en positius febles fins un 82% en positius forts.

Conclusions

A l'espera dels resultats definitius, els ANUC, especialment a títols elevats, semblen ser millor indicadors de LES que els anti-nDNA. La tècnica del dot-blot aporta resultats fiables i comparables als reportats en la literatura amb altres tècniques, però podria millorar-se usant un extracte lliure d'histona H1. Finalment, les dades obtingudes aconsellen incloure la determinació dels ANUC a les guies clíniques per a l'estudi inicial d'una sospita clínica de LES.

Posters
Clinical Immunology 11 - 18

The authors will attend the poster on **20/11/2014**: 17:30h; **21/11/2014**: 11:00h, 13:30h, 16:55h.

17 Evaluation of an automated system for the detection of autoantibodies by indirect immunofluorescence on HEp-2 cells

Laura Rubio[1]; Odette Viñas[1]

[1]*Immunology Department, Hospital Clinic, Barcelona*

INTRODUCTION: Evaluation of autoantibodies is critical in the serological diagnosis and follow-up of many autoimmune diseases. In regards as screening of autoantibodies against the cell nuclei (ANA) the recommended method is indirect immunofluorescence (IIF) on human epithelial (HEp-2) cells. However, visualisation is subjective as well as time consuming, therefore the need for automation and standardization of IIF evaluation.
The aim of this study was to evaluate the diagnostic performance of an automated IIF reading system compared with visual interpretation of IIF by experienced personnel.

METHODS: A cohort of 133 consecutive samples from different patients with suspected autoimmune diseases submitted to a university diagnostic immunology laboratory were analysed using the automated Image Navigator® system (Palex Medical S.A) on HEp-2000 cells substrate (according to the manufacturer instructions) and compared to the visual diagnostic evaluation of autoantibodies on HEp-2 cells (slides processed with a higher concentration of the anti-human-IgG-FITC conjugated reagent) used at the laboratory. 61 samples were assessed for dsDNA by ELISA, 60 for extractable nuclear antigens (ENA) by CLIA and 31 for other specificities by immunoblot, total 90 patients, according to the clinical requests.

RESULTS: Agreement in the discrimination of negative from positive samples between visual interpretation (17 negatives) and automated reading by the Image Navigator® (31 negatives) was fair (k= 40.11% (CI 21.16% - 59.06%)), the discrepancy corresponding to 24 patients, one of which had positive specificities to Jo-1 and Ro52. This difference in negative discrimination could be due to the different processing of the slides.
Interpretation of the pattern of the 102 positive samples was concordant for 81 (79.41%). Of the discrepant 21, 11 (52.38%) were either homogenous or speckled, due to the presence or not of condensed chromatin in the Hep-2000 cells. One of the 9 patients out of the 11 that had anti-dsDNA determined was positive. 12 of 21 had a discrepant nucleolar pattern, none of the 6 that had ENA tested were positive. 90 patients had confirmatory tests, 15 of them were positive for at least one specificity. Of those, the visual and automated reading was very similar but for one case (Jo-1 and Ro52), assigned as negative in the Image Navigator® system.

CONCLUSION: The discrepancies in negative from positive discrimination, and in pattern interpretation could be due largely to the different processing of the slides, increasing the fluorescence intensity of the HEp-2 slides, and the different substrates used in the study, respectively. However, these differences were no clinically significative. Overall, the Image Navigator® system was easy to use, allowed for review and tracing of the results, and saved time.

Posters
Clinical Immunology 11 - 18

The authors will attend the poster on **20/11/2014**: 17:30h; **21/11/2014**: 11:00h, 13:30h, 16:55h.

18 Comparació de diferents mètodes per determinar auto-anticossos anti-dsDNA

G. Julià[1]; C. Hernández[1]; E. Moltó[1]; C. Roldán[2]; L. Martínez-Martínez[1]; C. Gelpí[2].

[1]Hospital de la Santa Creu i Sant Pau; [2]Institut de Recerca Biomèdica Sant Pau

La presència d'auto-anticossos (auto-Ac) es un paràmetre fonamental per al diagnòstic de les malalties autoimmunes (AI). Tal es el cas de la detecció dels anticossos anti-nuclears (ANAs), anti-dsDNA, anti-Sm i anti cardiolipines als malalts de Lupus eritematós sistèmic (LES).

En el present treball hem estudiat la presència d'auto-Ac anti-dsDNA en 110 malalts diagnosticats de LES i 40 controls (individus sans o amb altres malalties no relacionades). Es van utilitzar 5 mètodes diferents per comparar la seva sensibilitat i especificitat: Radioimmunoassaig (RIA) anti-ds-DNA, el procediment de rutina del Hospital Sant Pau (PRHSP) que es basa en l'ús conjunt de dues tècniques (ELIA i IFI Crithidia luciliae), ELISA anti-dsDNA d'elaboració pròpia, immunofluorescència indirecta (IFI) sobre Crithidia luciliae i assaig de quimioluminescència (CLIA). L'analisi estadistic es realitzà mitjançant corbes ROC (Receiver Operating Characteristic).

Dels resultats obtinguts al nostre grup d'estudi, observem que tots els mètodes analitzats presenten valors acceptables de sensibilitat i especificitat per a la detecció de auto-Ac anti-dsDNA. El mètode que ofereix la màxima especificitat i sensibilitat es el PRHSP.

La detecció dels auto-Ac anti-dsDNA ha de fer front al problema de la gran diversitat i policlonalitat d'aquests auto-Ac, al fenomen de "epitop spreading" així com a la fluctuació en el seu títol. L'ús combinat de dues tècniques millora la sensibilitat i especificitat en la detecció dels auto-Ac anti-dsDNA.

Posters
Adaptative Immunity 19 - 21

The authors will attend the poster on **20/11/2014**: 17:30h; **21/11/2014**: 11:00h, 13:30h, 16:55h.

19 Splenic MAdCAM-1$^+$ Marginal Reticular Cells Have Stromal Properties and Deliver Antibody-Inducing Signals to Marginal Zone B Cells

Sabrina Bascones[1]; Giuliana Magri[1]; Linda Cassis[1]; Laura Comerma[1]; Carolina M. Barra[1]; Maurizio Gentile[1]; Irene Puga[1]; Andrea Cerutti[1,2]

[1]Institut Hospital del Mar d'Investigacions Mèdiques, Barcelona, Spain; [2]Immunology Institute, Mount Sinai School of Medicine, New York, USA.

The splenic marginal zone (MZ) generates prompt antibody responses to blood-borne antigens through a unique subset of innate-like B cells that closely interact with poorly characterized stromal cells. We found that human MZ contained a meshwork of fibroblast-like marginal reticular cells (MRCs) that predominantly developed after birth and expressed mucosal addressin cell adhesion molecule-1 (MAdCAM-1), a mucosal vascular addressin usually associated with intestinal endothelial cells. In general, MAdCAM-1 interacts with α4β7, a gut-homing receptor usually induced by intestinal lymphocytes in response to retinoic acid (RA), a derivative of dietary vitamin A. Unlike endothelial cells, MAdCAM-1$^+$ MRCs expressed stromal molecules such as Thy-1 (CD90), thrombomodulin (CD141), ICAM-1 and VCAM-1, but lacked endothelial molecules such as PECAM-1 (CD31), CD34 and von Willebrand factor. Similar to stromal lymphoid tissue organizers, MAdCAM-1$^+$ MRCs up-regulated the adhesion molecules ICAM-1 and VCAM-1 in response to the lymphoid tissue-inducing cytokines lymphotoxin and TNF. Moreover, MAdCAM-1$^+$ MRCs up-regulated the B cell-stimulating factors BAFF and APRIL in response to microbial TLR ligands and thereby delivered robust survival, proliferation, class switching and plasma cell differentiation signals to MZ B cells. Signals from TLRs further elicited human MRC activation of retinaldehyde dehydrogenase (RALDH) and production of RA, which induced the gut-homing receptors α4β7 and CCR9 on MZ B cells. These findings indicate that MRCs have unique B cell-helper properties that could be harnessed to enhance vaccine-induced antibody responses systemically and perhaps at mucosal sites of antigen entry.

Posters
Adaptative Immunity 19 - 21

The authors will attend the poster on **20/11/2014**: 17:30h; **21/11/2014**: 11:00h, 13:30h, 16:55h.

20 Caracterització fenotípica de ratolins deficients en CD6

Esther Carreras[1]; Marc Orta[1]; Inês Simões[1]; Vanesa G. Martínez[1]; Amado Carreras Sureda[2]; Miguel Valverde[2]; Ruben Vicente[2]; Adelaida Sarukhan[1]; Francisco Lozano[1]

[1]Institut d'Investigacions Biomédiques August Pi i Sunyer (IDIBAPS), Barcelona; [2]Laboratory of Molecular Physiology and Channelopathies, Department of Experimental and Health Sciences. Universitat Pompeu Fabra, Barcelona.

La glicoproteïna CD6 és un receptor limfocitari expressat principalment a cèl·lules T i a una subpoblació de cèl·lules B (B1a) i NK, encara que també es troba a precursors hematopoètics i algunes zones del cervell. CD6 participa per un costat en la estabilització la sinapsis immunològica a través de la unió al seu lligand ALCAM, una molècula d'adhesió expressada en cèl·lules presentadores d'antigen i per d'altre a la modulació del senyal del TCR durant els processos de maduració i activació limfocitaria. Donat que hi ha dades contradictòries sobre si aquest paper modulador és positiu o negatiu, en aquest treball s'ha caracteritzat les conseqüències de la deficiència de CD6 ($CD6^{-/-}$) sobre diferent aspectes de la fisiologia limfocitaria en un model murí en fons C57Bl/6. El ratolins $CD6^{-/-}$ son viables, sans i es comporten i reprodueixen normalment. En comparació amb els seus germans wild-type (WT), els ratolins $CD6^{-/-}$ tenen un menor nombre de timòcits i aquests són hiper-reactius a l'estimulació via TCR/CD3. Pel que fa a les subppoblacions limfocitàries, els ratolins $CD6^{-/-}$ tenen una proporció disminuïda de cèl·lules $TCD4^{+}$ tan en timus com en perifèria. Pel contrari, tenen un percentatge relatiu elevat de cèl·lules nTreg en timus i de T ($CD4^{+}$ i/o $CD8^{+}$) efectores i memòria en perifèria. La proporció de la resta de poblacions limfocitaries analitzades es similar en WT i $CD6^{-/-}$. En conclusió, els resultats obtinguts sóncompatibles amb un paper rellevant del receptor limfocitari CD6 en la homeòstasis d'algunes poblacions linfocitàries molt probablement com a conseqüència del seu efecte modulador negatiu sobre la senyalització del TCR.

Posters
Adaptative Immunity 19 - 21

The authors will attend the poster on **20/11/2014**: 17:30h; **21/11/2014**: 11:00h, 13:30h, 16:55h.

21 Down-modulation of CD6 surface expression following T cell activation

Esther Carrasco[1]; Cristina Escoda-Ferran[1]; Esther Carreras[1]; Marina Mané[1]; Miguel Caballero-Baños[2]; Cristina Miró-Julià[1]; Núria Climent[3]; Francisco Lozano[1,3,4]

[1]Institut d'Investigacions Biomèdiques August Pi i Sunyer (IDIBAPS), Centre Esther Koplowitz, Barcel; [2]Servei d'Immunologia, Centre de Diagnòstic Biomèdic, Hospital Clínic de Barcelona, Barcelona; [3]IDIBAPS-AIDS Research Group, HIVACAT, Barcelona; [4]Departament de Biologia Cel•lular, Immunologia i Neurociències, Facultat de Medicina, Universitat de Barcelona.

CD6 is a signal-transducing lymphocyte surface receptor expressed mainly in mature T lymphocytes, NK cells, and B1a lymphocytes. Based on its interaction with CD166/ALCAM and its physical association with the TCR/CD3 complex, CD6 has been involved in T-cell adhesion phenomena as well in modulation of T cell activation and developmental processes, respectively. Previous work have reported that, under some circumstances such as bone marrow transplantation or herpes virus infections, a CD6 negative T cell population emerges, whose nature and functional capabilities have not been explored. Here, we show that different T-cell activation stimuli (PHA, anti-CD3 plus anti-CD8, or allogenic mature dendritic cells) lead to a significant decrease in CD6 cell surface expression, which inversely correlated with an increase in CD25 expression. Furthermore, $CD6^-$ cells were found in $CD4^+CD25^+$ cells irrespective of their $FoxP3^+$ expression. The reduction of membrane CD6 was followed by the *in vitro* release of a soluble form of CD6, which could be partially blocked by ADAM protease inhibitors. Moreover, we found that the resultant $CD6^-$ T cell population showed higher apoptosis levels and impaired proliferation than $CD6^+$ T population, which is in agreement with previously reported anti-apoptotic properties of CD6. Our findings indicates that down-modulation of CD6 surface levels following T-cell activation could play a relevant role in the termination and/or homeostasis of immune responses.

*Work supported by Plan Nacional de I+D+i, Ministerio de Economia y Competitividad (SAF 2010-19717 i SAF 2013-46151-R).

2014 Events

Lifelong Learning SCI Program

Data i hora: 5 de febrer de 2015, a les 18:30h.
"**Microinflamación local y disfunción intestinal: avances en la fisiopatología del Síndrome del Intestino Irritable**".
Dra. Maria Vicario (Neuro-immuno-gastroenterology Lab. Digestive Diseases Research Unit. Vall d'Hebron Institut de Recerca. Barcelona). Lloc: Sales Polivalents, Planta 2, de Hospital de la Santa Creu i Sant Pau (Sant Antoni Maria Claret, 167, Barcelona).

Data i hora: 5 de març de 2015, a les 18:30h.
"Por determinar".
Dr. Miguel López-Botet (PRBB. UPF Hospital del Mar. Barcelona). Lloc: Auditori Acadèmia de Ciències Mèdiques (Major de Can Caralleu 1-7, Barcelona).

Data i hora: 9 d'abril de 2015, a les 18:30h.
"TCR immunodeficiencies".
Dr. Jose Ramón Regueiro (Departamento de Microbiologia I-Inmunología, Uinversidad Complutense de Madrid). Lloc: Parc de Recerca Biomèdica de Barcelona (PRBB), sala Xipre 1ª planta (c/Doctor Aiguader 88, Barcelona).

Data i hora: 29 d'abril de 2015, dia de la IMMUNOLOGIA, 16h-21h
Tema: APORTACIÓ DE LES ÒMIQUES A LA IMMUNOLGIA
Una iniciativa de la IUIS (International Union of Immunological Societies) amb la col·laboració directa de la EFIS (European Federation of Immunological Societies). Lloc: Institut d'Estudis Catalans, sala Pere i Joan Coromines. c/del Carme 47. Barcelona.

Data i hora: 7 de maig de 2015, a les 18:30h.
"Microvesicules i resposta immunitària".
Dr. Francesc Borràs (IVECAT Group Leader. Institut d'Investigació Germans Trias i Pujol. Badalona. Barcelona). Lloc: Hospital Clinic, Sala Farreras Valentí, escales 9-11, (c/Villarroel 170, Barcelona).

Data i hora: 4 de juny de 2015, a les 18:30h.
"Factors de transcripció en macròfags".
Dr. Jorge Lloberas (Departament de Fisiologia i Immunologia. Parc Cientific de Barcelona, Universitat de Barcelona). Lloc: Auditori Acadèmia de Ciències Mèdiques (Major de Can Caralleu 1-7, Barcelona).

Data i hora: 2 de juliol de 2015, a les 18:30h.
"Influenza virus in pigs".
Dr. Maria Montoya (Investigadora CRESA-UAB, The Pirbright Institute, Londres). Lloc: Parc de Recerca Biomèdica de Barcelona (PRBB), sala Xipre 1ª planta (c/Doctor Aiguader 88, Barcelona).

New members

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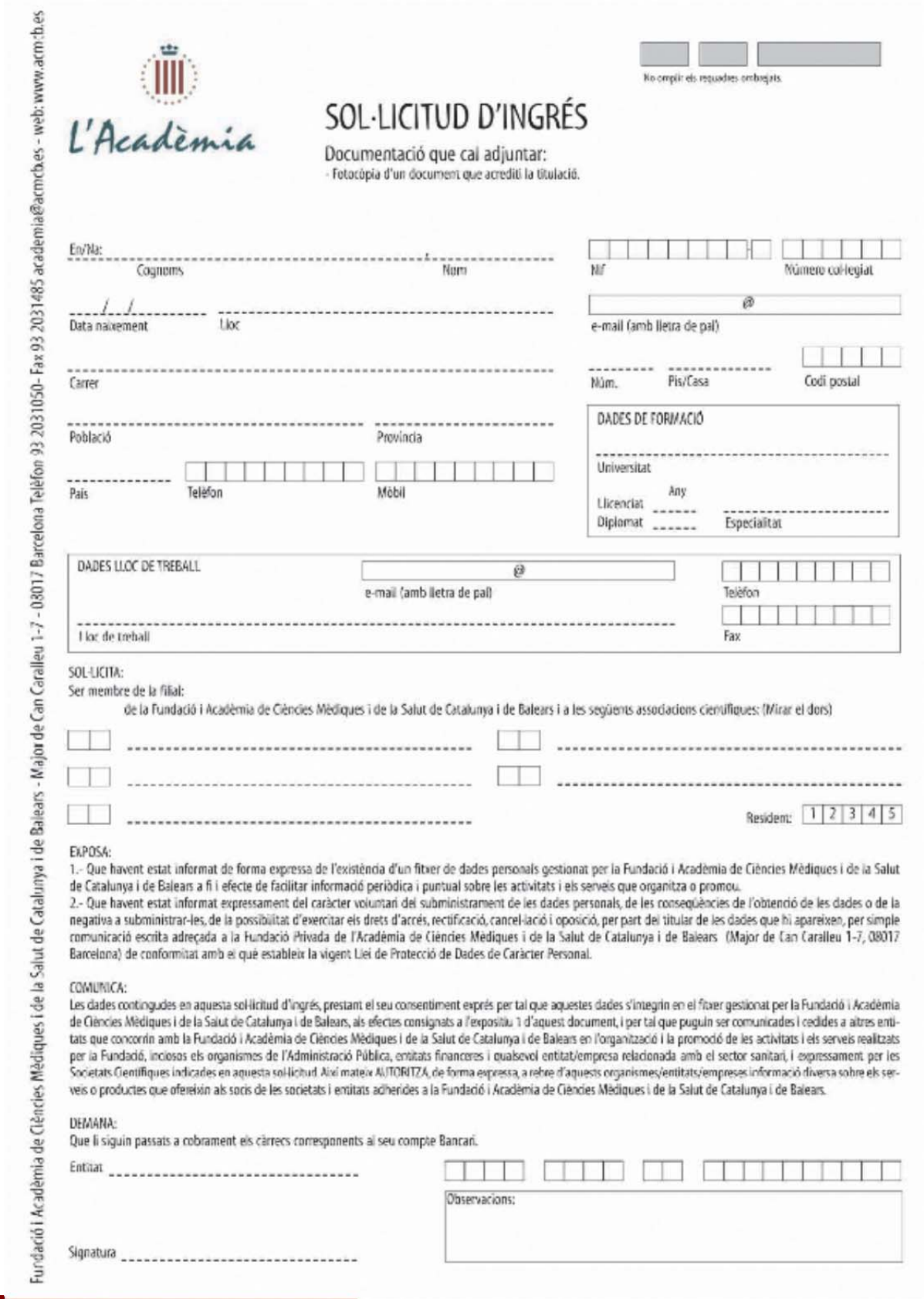

Fundació i Acadèmia de Ciències Mèdiques i de la Salut de Catalunya i de Balears - Major de Can Caralleu 1-7 - 08017 Barcelona Telèfon 93 2031050 - Fax 93 2031485 academia@acmcb.es - web: www.acmcb.es

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Les dades contingudes en aquesta sol·licitud d'ingrés, prestant el seu consentiment exprés per tal que aquestes dades s'integrin en el fitxer gestionat per la Fundació i Acadèmia de Ciències Mèdiques i de la Salut de Catalunya i de Balears, als efectes consignats a l'expositiu 1 d'aquest document, i per tal que puguin ser comunicades i cedides a altres entitats que concorrin amb la Fundació i Acadèmia de Ciències Mèdiques i de la Salut de Catalunya i de Balears en l'organització i la promoció de les activitats i els serveis realitzats per la Fundació, incloses els organismes de l'Administració Pública, entitats financeres i qualsevol entitat/empresa relacionada amb el sector sanitari, i expressament per les Societats Científiques indicades en aquesta sol·licitud. Així mateix AUTORITZA, de forma expressa, a rebre d'aquests organismes/entitats/empreses informació diversa sobre els serveis o productes que ofereixin als socis de les societats i entitats adherides a la Fundació i Acadèmia de Ciències Mèdiques i de la Salut de Catalunya i de Balears.

DEMANA:
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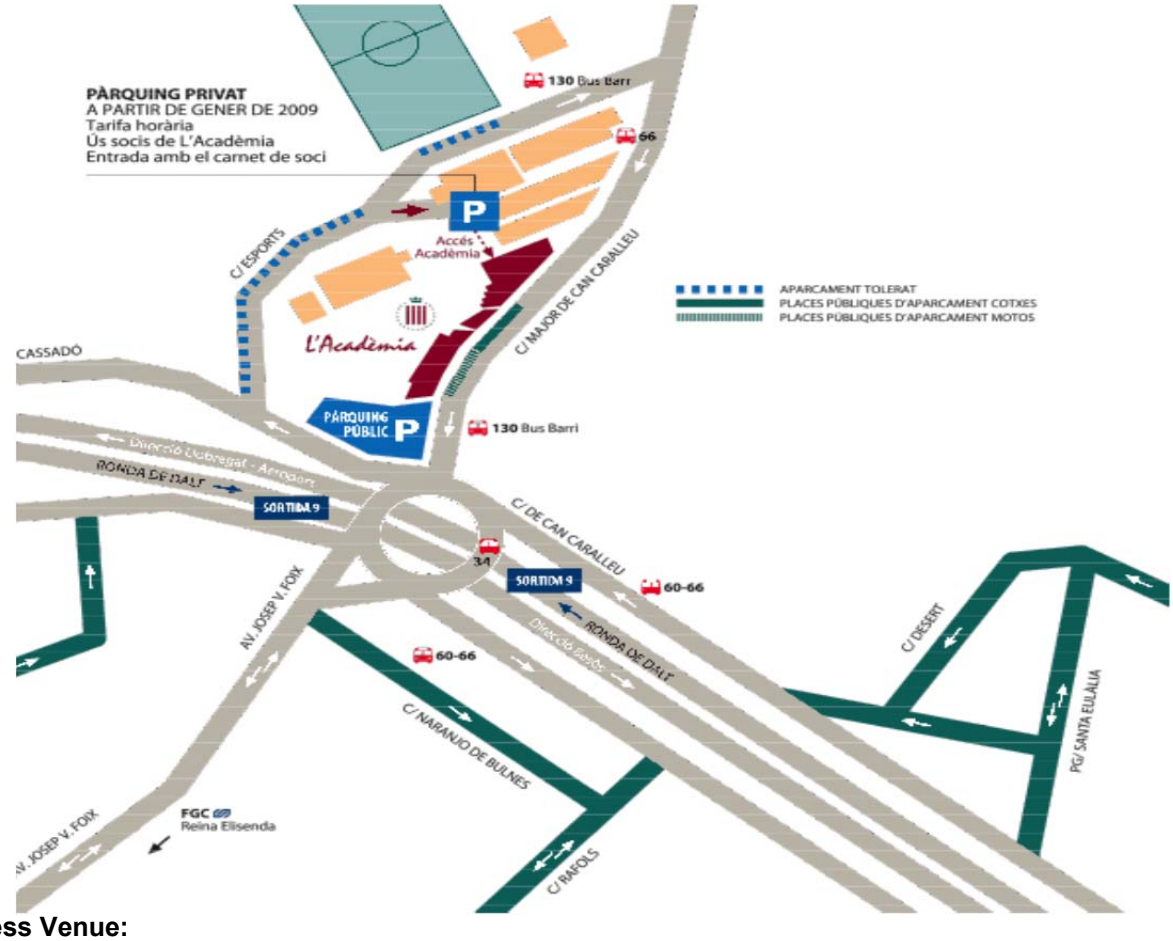
Entitat

Observacions:

Signatura

Participant information

Useful information



Congress Venue:
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Transportation:

- **By car**: Ronda de Dalt, exit 9
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  - Line 60 (Pl. Glòries – Zona Universitaria)
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  - Line 130 (Pl. Artós – Can Caralleu))
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Parking: Small open area between the Academy and the city ring (only for members of the Academy: Parking fees apply).

Congress Office:

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Tel: 00 34 93.203.13.18FAX: 00 34 93 212 35 69
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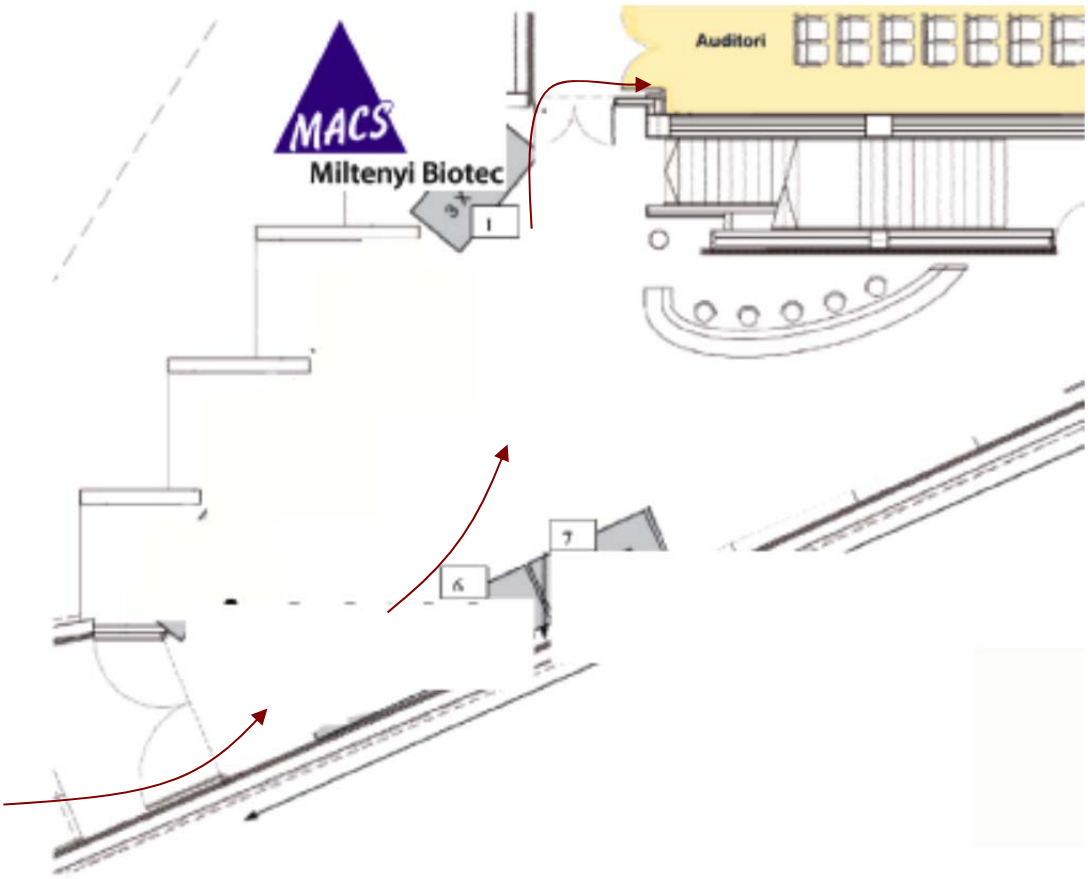
List of participants and authors

Premi a la millor comunicació oral i al millor pòster en el VIIIè Congrés SCI:

Per cinquè any consecutiu s'atorgarà també el premi a la millor comunicació oral (500 €) i al millor pòster (250 €) del present congrés. El comité cientific i la Junta de la SCI seleccionaran entre les comunicacions presentades en cada categoria aquella que destaca tant pels seus valors cientícs com per aspectes relacionats amb la pròpia presentació. Les resolucions es faran públiques a la fi del congrés.

Other useful information and notes



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VIII CONGRÉS

Societat Catalana d'Immunologia (SCI)

Barcelona, 20 i 21 de novembre 2015

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