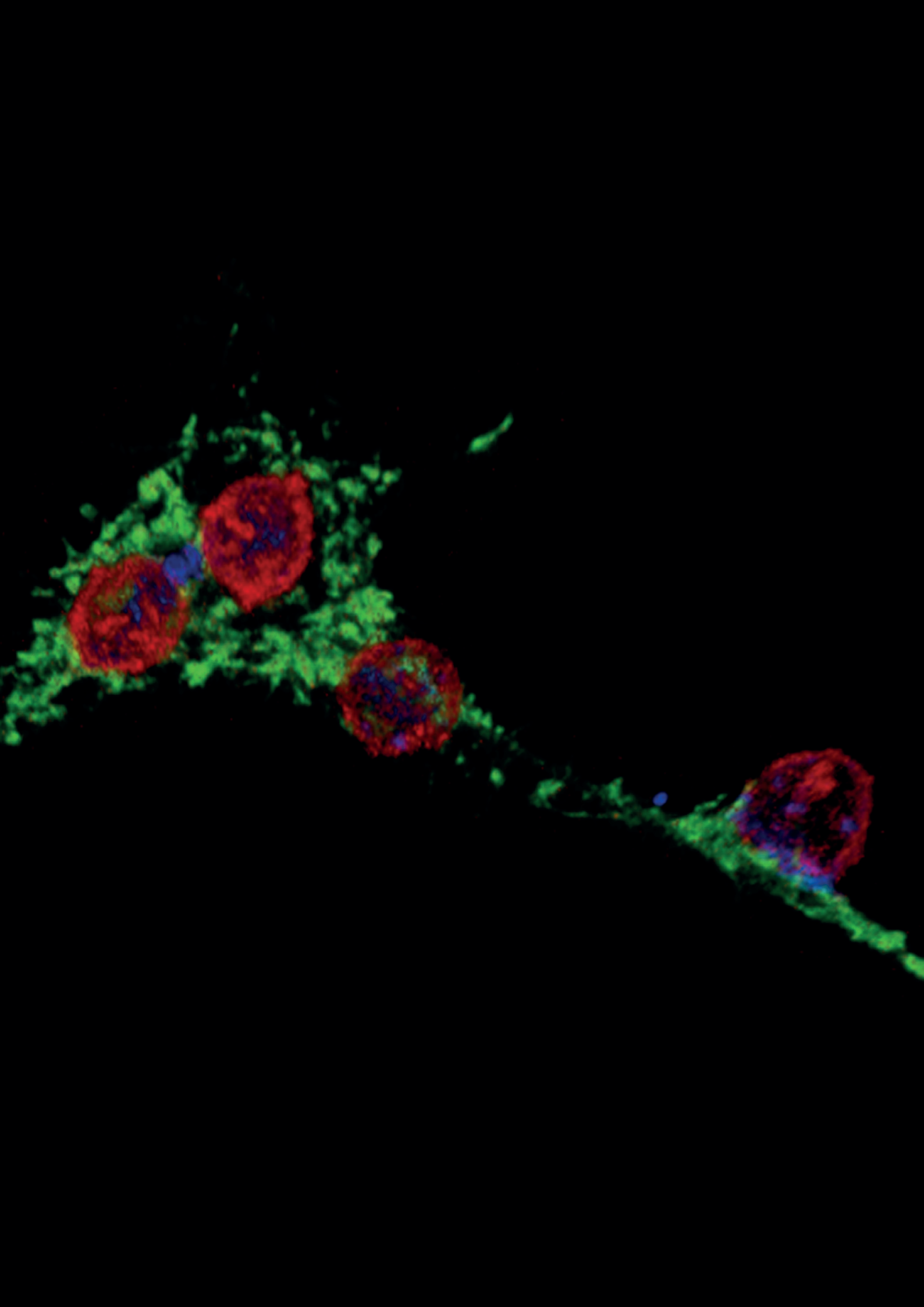

5

IMMUNOLOGY AND ONCOLOGY

Research carried out in the Department of Immunology and Oncology (DIO) centres on characterizing the molecular and cellular bases of immune system function in inflammation and tumour development. Our general objective is to develop improved or new approaches for modulating the immune response during inflammatory reactions. We thus aim to identify new targets for prevention, diagnosis and treatment of inflammatory or autoimmune diseases and cancer.

The various research groups in the department address the cellular and molecular mechanisms of innate and adaptive immunity in inflammation and cancer. Our interests focus on inflammation in different pathological contexts such as infection, autoimmunity, inflammation-driven carcinogenesis, as well as tumour immunology and stem cells. Based on this knowledge, we work to develop new tools for immunotherapy.

From a methodological perspective, the department's activities are multidisciplinary. Our expertise combines cutting-edge technologies such as advanced microscopy (total internal reflection fluorescence microscopy or real-time confocal microscopy), multi-parameter flow cytometry, nanoparticle production, and next-generation "omics". In addition, we have extensive experience in the generation and use of genetically modified mouse models using the latest techniques.

Our common research objective provides an excellent environment and numerous opportunities for collaboration within the department as well as with other groups in the CNB (for example, the nanobiomedicine initiative). Since its origins, the DIO has maintained stable, productive collaborations with public and private partners that include prominent national and international research institutes, hospitals and pharmaceutical companies.

From an operational view, the DIO holds weekly seminars and a scientific retreat each year. These events promote fruitful scientific discussion and interaction among the department members and provide an atmosphere that fosters new collaborations. The DIO also holds bi-weekly *Friends of the DIO Seminars*, in which colleagues from other institutes present their current research results. Our seminars form part of the curriculum for the Master's degree in Immunology (Universidad Complutense de Madrid).

HEAD OF DEPARTMENT

Ana Cuenda

OUR RESEARCH GROUPS

1. **Dendritic cell immunobiology**
   Carlos Ardavín
2. **Deactivation and reactivation of the immune response in autoimmunity and cancer**
   Dimitrios Balomenos
3. **Nanomedicine, cancer immunotherapy and autoimmune diseases**
   Domingo F. Barber
4. **Cardiac stem cells**
   Antonio Bernad
5. **B cell dynamics**
   Yolanda R. Carrasco
6. **Molecular targets in health and disease: focus on PI3-kinase**
   Ana Clara Carrera
7. **Role of stress-activated protein kinase p38MAPK in human disease**
   Ana Cuenda
8. **Physiopathology of chemokine receptor interactions**
   Leonor Kremer
9. **Signalling networks in inflammation and cancer**
   Santos Mañes
10. **Stem cells and immunity**
    Carlos Martínez-A
11. **Chemokine receptors: new targets for therapeutic intervention**
    Mario Mellado
12. **Diacylglycerol kinases in the control of immune response and cancer progression**
    Isabel Mérida
13. **Transcriptional control of B lymphocyte differentiation**
    Ignacio Moreno de Alborán
14. **Receptor-ligand interactions in immune responses to cancer and viruses**
    Hugh T. Reyburn
15. **T cell signalling in autoimmune diseases and cancer**
    Jesús M. Salvador
16. **Tumour immune activation and evasion**
    Mar Valés-Gómez

B lymphocytes (labeled for B220; red) establishing the immune synapse with a target cell (labeled for ICAM-1; green) that exposed pathogen-derived antigens (labeled in blue) on its cell surface

Dendritic cell immunobiology

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SELECTED PUBLICATIONS

Martínez del Hoyo G, Ramírez-Huesca M, Levy S, Boucheix C, Rubinstein E, Minguito de la Escalera M, González-Cintado L, Ardavín C, Veiga E, Yáñez-Mó M, Sánchez-Madrid F. CD81 controls immunity to *Listeria* infection through Rac-dependent inhibition of proinflammatory mediator release and activation of cytotoxic T cells. J Immunol 2015; 194: 6090-101

Sánchez Vallecillo MF, Minguito de la Escalera M, Ullio Gamboa GV, Palma SD, González-Cintado L, Chiodetti AL, Aguirre MV, Soldano G, Morón G, Allemandi DA, Ardavín C, Pistoresi-Palencia MC, Maletto BA. A liquid crystal of ascorbyl palmitate, used as vaccine platform, has intrinsic pro-inflammatory and adjuvant activity which are dependent on MyD88 adaptor protein. J Control Release 2015 214: 12-22

Our research programme aims at exploring the role of inflammatory monocytes and macrophages during infection, allergy and intraperitoneal tumour metastasis, and encompasses the following research lines:

- Role of monocytes and type-I interferon in NK cell and neutrophil activation during the innate immune response against systemic *Candida albicans* infection
- Alveolar macrophage dynamics and immunophysiology during airway allergic reactions caused by house dust mite-derived allergens
- Role of the innate immune system of the peritoneal cavity in defence against intraperitoneal bacterial infections and colorectal tumour metastasis

The experimental approach designed to address the role of monocytes during Candida infection involves analysis of the early and cooperative spleen and kidney innate immune responses to intravenous infection with the fungus *Candida albicans* in a mouse model of systemic candidiasis, using wild type mice of the C57BL/6 strain and mice deficient in the type-I interferon receptor (IFNAR), the chemokine receptor CCR2, and the cytokine IL-15.

Our project on the dynamics of alveolar macrophages involves the study of the alveolar damage caused by strong airway allergic reactions against house dust mite-derived allergens, the process by which the alveolar macrophage subset is regenerated once the allergic process is resolved, and the mechanisms ensuring alveolar tissue repair and surfactant homeostasis. Wild type C57BL/6 and CCR2-deficient mice, parabiosis and progenitor transfer experiments, as well as immunofluorescent and electron microscopy are currently used in our laboratory to address these issues.

Experimental sepsis, peritoneal bacterial infection models, using mouse intestinal strains of *Escherichia coli* and mouse models of intraperitoneal metastasis of colorectal tumours in wild type C57BL/6 and CCR2-deficient mice, are used to explore the role of the innate immune system of the peritoneal cavity in defence against intraperitoneal infection and tumour metastasis.

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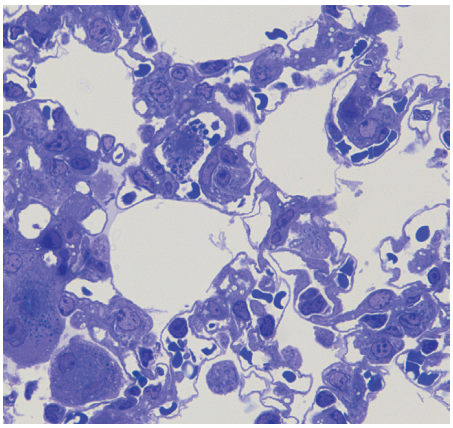

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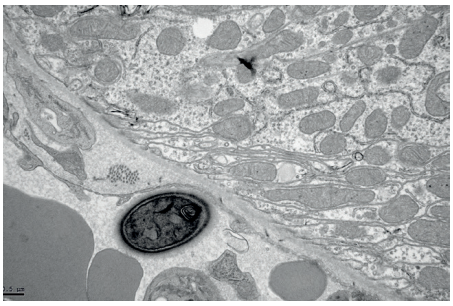
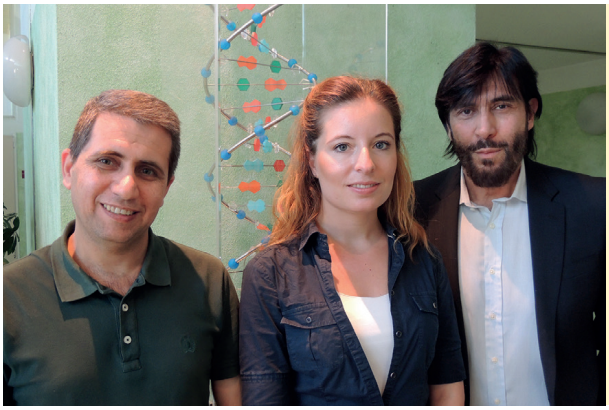


1
Alveolar damage and pneumocyte hyperplasia caused by house dust mite airway allergy. Light microscopy of a 1 µm semithin section of lung 4 days after intratracheal allergen challenge.

2
Invasion of a renal proximal convoluted tubule epithelium by a Candida yeast. Electron microscopy of an ultrathin section of kidney 18 h after intravenous infection.



Deactivation and reactivation of the immune response in autoimmunity and cancer

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SELECTED PUBLICATIONS

Daszkiewicz L, Vázquez-Mateo C, Rackov G, Ballesteros-Tato A, Weber K, Madrigal-Avilés A, Di Pilato M, Fotedar A, Fotedar R, Flores JM, Esteban M, Martínez-A C, Balomenos D. Distinct p21 requirements for regulating normal and self-reactive T cells through IFN-gamma production. Sci Rep 2015; 5: 7691

Rackov G, Hernández-Jiménez E, Shokri R, Carmona-Rodríguez L, Mañes S, Álvarez-Mon M, López-Collazo E, Martínez-A C, Balomenos D. p21 mediates macrophage reprogramming through regulation of p50-p50 NF-κB and IFN-β. J Clin Invest 2016; 126: 3089-103

The immune system is indispensable for defence against microbial invaders, and T cells and macrophages are essential components of protective immunity.

In autoimmune diseases, hyperactivated immunity provokes destructive self-reactivity. To neutralize this damaging effect, the immune response must be deactivated. In contrast, in cancer, immunosuppressed immunity requires reactivation. Our studies show that p21 is a regulator of the balance between hyperactivation and immunosuppression, and could control autoimmunity and cancer.

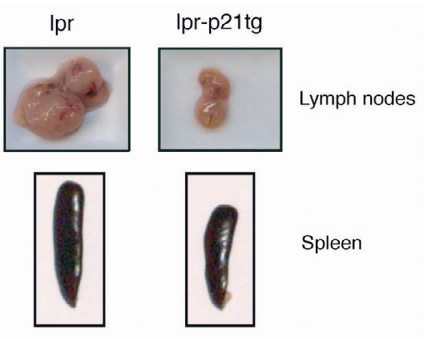
Novel p21 functions in deactivating autoimmune T memory responses

Compared to normal memory T cells, autoreactive T cells are overactivated by their repeated encounters with autoantigens. We recently showed therapeutic potential for p21, as its overexpression deactivates hyperactivated autoreactive T cells, but does not affect normal protective immunity (Daszkiewicz *et al.,* 2015). Our current analysis indicates that p21 does not act as a cell cycle inhibitor, but limits the activation of autoreactive T cells. We consider that, by deciphering the pathway by which p21 suppresses T cell activation, our studies will uncover new ways to deactivate autoreactive T cells and treat autoimmunity.

p21 modulates M1-to-M2 macrophage reprogramming in LPS tolerance: effects in cancer immunotherapy

We previously found that lack of p21 intensifies septic shock. Sepsis in humans is due not only to septic shock, but also to the macrophage unresponsiveness that follows shock. We recently showed that p21 drives this unresponsive state. M1-to-M2 macrophage reprogramming develops during LPS tolerance, a state refractory to LPS rechallenge that resembles sepsis hyporesponsiveness. Lack of p21 prevents macrophage reprogramming to M2 status (Rackov et al., 2016). This might have a direct effect in cancer immunotherapy, as tumor persistence neutralizes M1 macrophages and attracts deactivated M2 cells. As p21 controls M1-to-M2 reprogramming and p21-/- macrophages remain in the M1 state, we predict anti-tumor potential for p21-/- macrophages.

1



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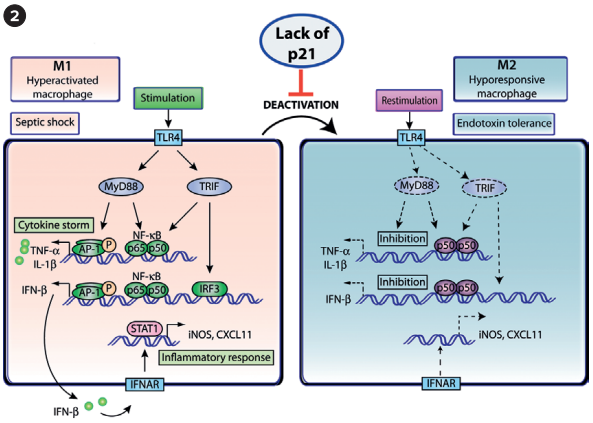
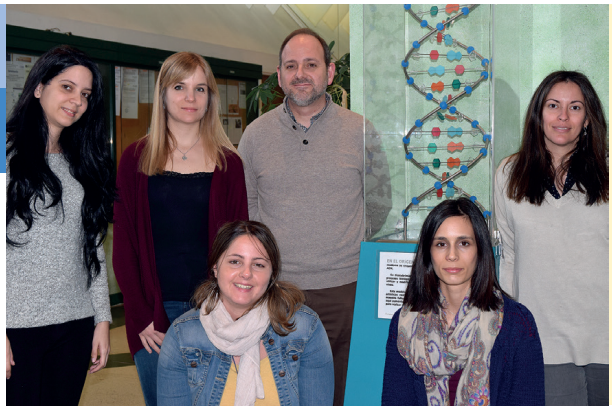


1 p21 overexpression reduces autoimmune T cell activation and expansion in lymph node and spleen

2 Lack of p21 prevents macrophage deactivation and hyporesponsiveness



Nanomedicine, cancer immunotherapy and autoimmune diseases

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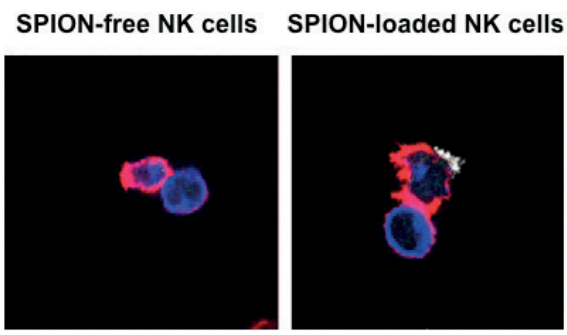
José Luis Tajada Herráiz

Besides its physiological functions –identifying and eliminating harmful pathogens without harming the tissues and organs themselves— the immune system can be manipulated specifically for therapeutic purposes by active immunization, immunotherapy or immunomodulation. We refer to immunosuppressive immunotherapy when the objective is to reduce or suppress the body's immune response, as in the case of autoimmune diseases, and immunostimulatory immunotherapy when the objective is to elicit or amplify the natural immune response, as in the case of antitumor immunotherapy. Immunosuppressors or immunostimulants, including biomolecules and drugs, are already used to prevent and to treat numerous diseases. Their use is generally limited, however, due to the systemic toxicity they can produce. Traditional cancer treatments can likewise generate numerous side effects due to their systemic toxicity.

Our group considers that the use of nanoparticle-based nanomedicines in immunosuppressive or anti-tumour therapies will improve the effectiveness of existing therapies. It would permit the release of drugs or biomolecules specifically at the site of action, which could achieve high local concentrations, as the nanoparticles can be guided and focused on the area of interest by an external magnetic field and/or by functionalization, while maintaining low systemic concentrations that would reduce the undesirable side effects of current therapies. The combination of nanoparticles and immunomodulation seems a very attractive idea.

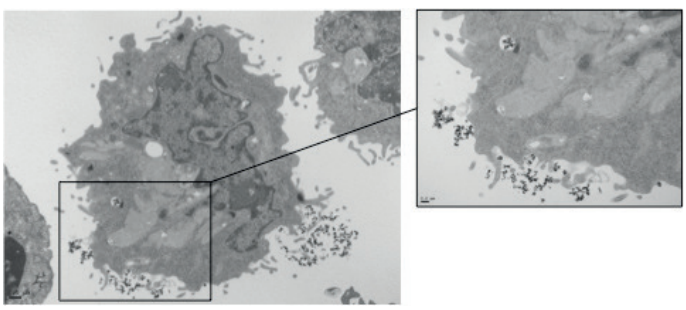
The overall objective of the group is to develop new nanoparticle-based nanomedicines that allow efficient, specific targeting of drugs, biomolecules or cell types to the desired site of action in anti-tumour and immunosuppressive therapies.

1



1 Conjugation of SPION-free and -loaded NK cells with target cells (RMA-S cells; 5 min), observed by confocal microscopy (Photo: L Sanz-Ortega)

2



2 Uptake of magnetic nanoparticles by natural killer (NK92MI) cells, observed by transmission electronic microscopy. (Photo: L Sanz-Ortega)

SELECTED PUBLICATIONS

Spada R, Rojas JM, Pérez-Yagüe S, Mulens V, Cannata-Ortiz P, Bragado R, Barber DF. NKG2D ligand overexpression in lupus nephritis correlates with increased NK cell activity and differentiation in kidneys but not in the periphery. J Leukoc Biol 2015; 97: 583-98

Mulens-Arias V, Rojas JM, Pérez-Yagüe S, Morales MP, Barber DF. Polyethylenimine-coated SPIONs trigger macrophage activation through TLR-4 signaling and ROS production and modulate podosome dynamics. Biomaterials 2015; 52: 494-506

Mulens-Arias V, Rojas JM, Pérez-Yagüe S, Morales MP, Barber DF. Polyethylenimine-coated SPION exhibits potential intrinsic anti-metastatic properties inhibiting migration and invasion of pancreatic tumor cells. J Control Release 2015; 216: 78-92

Rojas JM, Sanz-Ortega L, Mulens-Arias V, Gutiérrez L, Pérez-Yagüe S, Barber DF. Superparamagnetic iron oxide nanoparticle uptake alters M2 macrophage phenotype, iron metabolism, migration and invasion. Nanomedicine 2016; 12: 1127-38

Rojas JM, Spada R, Sanz-Ortega L, Morillas L, Mejías R, Mulens-Arias V, Pérez-Yagüe S, Barber DF. PI3K p85 β regulatory subunit deficiency does not affect NK cell differentiation and increases NKG2D-mediated activation. J Leukoc Biol 2016; 100: 1285-1296

Cardiac stem cells

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Rosa M. Carmona

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Ana Ortiz de Zárate

SELECTED PUBLICATIONS

Valiente-Alandi I, Albo-Castellanos C, Herrero D, Arza E, Garcia-Gomez M, Segovia JC, Capecchi M, Bernad A. Cardiac Bmi1+ cells contribute to myocardial renewal in the murine adult heart. Stem Cell Res Ther 2015; 6: 205

Cruz FM, Tomé M, Bernal JA, Bernad A. miR-300 mediates Bmi1 function in primitive cardiac progenitors and regulates differentiation. Cell Death Dis 2015; 6: e1953

Barreiro O, Cibrian D, Clemente C, Alvarez D, Moreno V, Valiente Í, Bernad A, Vestweber D, Arroyo AG, Martín P, von Andrian UH, Sánchez Madrid F. Pivotal role for skin trans-endothelial radio-resistant anti-inflammatory macrophages in tissue repair. eLife 2016; 5: e15251

Cañón S, Caballero R, Herraiz-Martínez A, Pérez-Hernández M, López B, Atienza F, Jalife J, Hove-Madsen L, Delpón E, Bernad. A miR-208b upregulation interferes with calcium handling in HL-1 atrial myocytes: Implications in human chronic atrial fibrillation. J Mol Cell Cardiol 2016; 99: 162-73

Valiente-Alandi I, Albo-Castellanos C, Herrero D, Sanchez I, Bernad A. Bmi1+ cardiac progenitor cells contribute to myocardial repair following acute injury Stem Cell Res Ther 2016; 7: 100

Mammalian heart can refresh aged or damaged cells during their lifetime, although at low rates. The current hypotheses for homeostatic turnover mainly involves the existence of a small population of stem/progenitor cells, combined with the potential de-differentiation of a minority population of mature cardiomyocytes. The nature and contribution to adult cardiac turnover of different proposed stem cell/progenitor populations continues to be debated. We have concluded that a population of non-cardiomyocytic cells, positive for Sca1 and that expresses high levels of the polycomb Bmi1 transcription factor (Bmi1^{h+}), contributes to the turnover of the three main cardiac lineages during homeostasis. In response to an acute myocardial infarct, the Bmi1^{h+} progeny substantially increase their contribution.

In adult tissues, progenitors and stem cells are lodged in niches that provide a low oxidative environment that is essential for correct regulation of their cell cycle status and metabolism. We found that Bmi1^{h+} cells show low levels of reactive oxygen species (ROS) and that BMI1 repressed cell fate genes, thereby favouring maintenance of the progenitor pool. Oxidative damage modifies BMI1 activity *in vivo*, derepressing canonical target genes and thus inducing proliferation and differentiation of cardiac progenitor cells. These findings demonstrate that the redox status influences the adult cardiac progenitor response and identify a redox-mediated BMI1 function with implications in adult cardiac turnover.

In parallel, we studied a human population (CPC; cardiac progenitor cells) currently being evaluated in a clinical trial (I/IIa) for the treatment of large acute myocardial infarcts using allogeneic cells. Using a combined proteomics and transcriptomics approach, we defined a panel of specific membrane markers as well as the selective members of secretome and exosomes. The clinical trial (CAREMI; NCT02439398), involving 55 patients, will finalize in April 2017.

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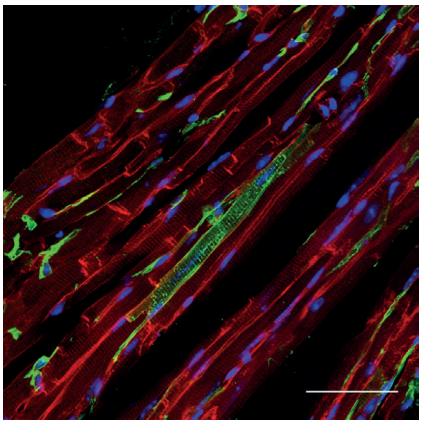
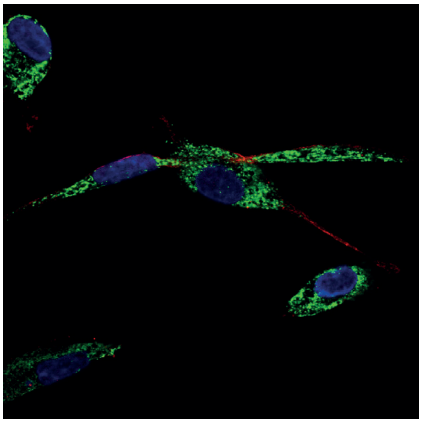


1 Cardiomyocytes derived from murine Bmi1^{h+} cells (green). Red indicates staining with cardiac sarcomeric actinin; nuclei (blue) were stained with DAPI.

2



2 Expression of CXCR1 (red) by human CPC cells. Green corresponds to laminin; nuclei (blue) were stained with DAPI.

B cell dynamics

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Robles EF, Mena-Varas M, Barrio L, Merino-Cortes SV, Balogh P, Du MQ, Akasaka T, Parker A, Roa S, Panizo C, Martin-Guerrero I, Siebert R, Segura V, Agirre X, Macri-Pellizeri L, Aldaz B, Vilas-Zornoza A, Zhang S, Moody S, Calasanz MJ, Tousseyn T, Broccardo C, Brousset P, Campos-Sanchez E, Cobaleda C, Sanchez-Garcia I, Fernandez-Luna JL, Garcia-Muñoz R, Pena E, Bellosillo B, Salar A, Baptista MJ, Hernandez-Rivas JM, Gonzalez M, Terol MJ, Climent J, Ferrandez A, Sagaert X, Melnick AM, Prosper F, Oscier DG, Carrasco YR, Dyer MJ, Martinez-Climent JA. Homeobox NKX2-3 promotes marginal-zone lymphomagenesis by activating B-cell receptor signaling and shaping lymphocyte dynamics. Nat Commun 2016; 7: 11889

Andrada E, Almena M, de Guinoa JS, Merino-Cortes SV, Liébana R, Arcos R, Carrasco S, Carrasco YR, Merida I. Diacylglycerol kinase ζ limits the polarized recruitment of diacylglycerol-enriched organelles to the immune synapse in T cells. Sci Signal 2016; 9: ra127.

Lymphocytes travel across tissue barriers and move through the interstitial space in response to external signals. This continuous migration throughout the stromal cell network allows the lymphocytes to seek pathogen-derived antigens in secondary lymphoid organs. Antigen recognition halts motile lymphocytes and leads to immune synapse (IS) formation with the antigen-presenting cell; this long-lasting cell-to-cell interaction is critical for lymphocyte activation. Lymphocytes are also exposed to a variety of non-cognate signals such as innate/inflammatory stimuli that modify cell motility/adhesion abilities and cell localization. Lymphocyte dynamics plasticity is thus intrinsic to lymphocyte function and crucial for adaptive immune protection. Knowledge of the molecular mechanisms that govern lymphocyte behaviour will reveal essential aspects of the immune response with potential therapeutic application.

We study how distinct stimuli (chemokines, antigen, innate signals) shape B lymphocyte dynamics to regulate cell function. We identified the molecular axis Syk/PIP_2/vinculin as a major regulator of B cell motility and stable adhesion in response to chemokine and antigen. During 2015-2016, we studied two proteins implicated in this axis, Bruton's tyrosine kinase (Btk) and diacylglycerol kinase (DGK). Btk has kinase and scaffold functions, both of which are similarly crucial for B cell motility; for IS establishment and assembly, however, we found the scaffold function to be more important than the kinase activity. DGK-zeta participated in IS formation but not in B cell motility. DAG, whose levels are controlled by DGK-zeta, regulates IS formation and cell activation; we found that DGK-zeta production of phosphatidic acid (PA) also governed assembly of the adhesive platform at the IS through allosteric modulation of PIP5KI (a PIP_2-producing lipid kinase) and regulation of the DOCK2/Rac axis. We also analysed changes associated with lymphomagenesis. Our findings in a marginal-zone lymphoma mouse model indicate that cell dynamics alterations in the whole polyclonal B cell population precede monoclonal B cell transformation and tumourigenesis.

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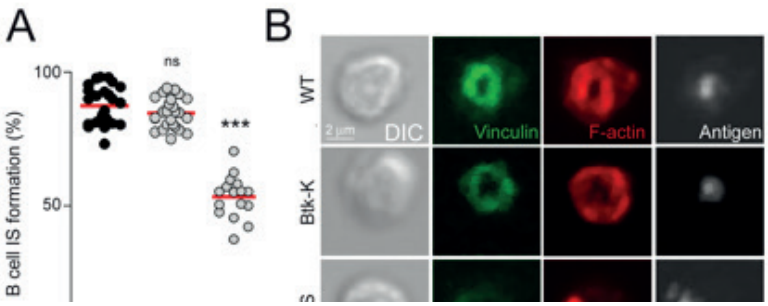

1

Distinct roles for the Btk scaffold and kinase activities in B cell synapse formation. (A) IS formation frequency of B cells with normal Btk (WT), that lack kinase activity (Btk-K) or are defective for scaffold activity (Btk-S); ns, not significant; *** p<0.0001. (B) DIC and fluorescence images for vinculin, F-actin and antigen at the contact plane of the mature IS of representative WT, Btk-K and Btk-S B cells.

Molecular targets in health and disease: focus on PI3-kinase

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SELECTED PUBLICATIONS

Redondo-Muñoz J, Pérez-García V, Rodríguez MJ, Valpuesta JM, Carrera AC. Phosphoinositide 3-kinase beta protects nuclear envelope Integrity by controlling RCC1 localization and Ran activity. Mol Cell Biol 2015; 35: 249-63

Millán-Uclés Á, Zuluaga S, Marqués M, Vallejo-Díaz J, Sanz L, Cariaga-Martínez AE, Real FX, Carrera AC. E-cadherin downregulation sensitizes PTEN-mutant tumors to PI3Kβ silencing. Oncotarget 2016; 7: 84054-71

Vallejo-Díaz J, Olazabal-Morán M, Cariaga-Martínez AE, Pajares MJ, Flores JM, Pio R, Montuenga LM, Carrera AC. Targeted depletion of PIK3R2 induces regression of lung squamous cell carcinoma. Oncotarget 2016; 7: 85063-78

Our laboratory focuses on the molecular mechanisms by which kinases control cell behaviour and, when altered, human disease. We concentrate on phosphoinositide 3-kinases (PI3-kinases). This enzyme generates the PIP3 product, which is increased in cancer and autoimmunity. PI3-kinases are heterodimers, with a p110 catalytic and a p85 regulatory subunit. p110alpha and beta, and associated p85alpha and p85beta, are ubiquitous and are altered in cancer. p110delta and the related p110gamma isoform are more abundant in haematopoietic cells; when deregulated, they participate in chronic inflammation and autoimmunity. We use mouse models to understand physiological PI3-kinase function and its role in human disease.

Mechanism of PI3-kinase beta action on DNA/chromatin remodelling. Many laboratories and companies have focused on PI3-kinase alpha; we study the less-known PI3-kinase beta. We showed that p110beta localizes to the nucleus and regulates DNA replication, segregation and repair; our objective is to understand the molecular basis of p110beta action on DNA homeostasis and chromatin remodelling.

Alternative cancer treatment based on interfering molecules. Metastasis remains the leading cause of death from many tumours. The PIP3 product enables cell survival; cells with altered regulation of PI3-kinase alpha or beta, or with mutations in phosphatase PTEN (which reduces PIP3 levels), are found in ~50% of human tumours, mostly in metastatic phases. PI3-kinase is a key target in cancer, but only broad-spectrum or p110alpha inhibitors are being tested clinically. We aim to develop a strategy to treat tumours accessible by endoscopy (or orally) based on delivery of interfering molecules.

New therapeutic targets in cancer. Although several recently developed inhibitors of frequently mutated proteins have improved prognosis of some cancers, mortality of other tumour types remains high. The Cancer Genome Atlas Project defines the most frequently altered genes in each tumour type; we study the less-known functions of some of these genes. This knowledge would permit definition of additional "driver mutations" and development of new therapeutic approaches.

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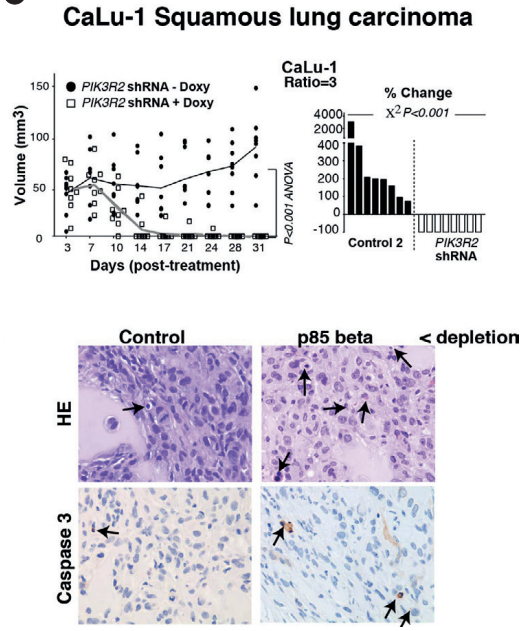

1

Squamous lung carcinoma response to p85beta depletion. Top, lung squamous cell line CaLu-1 expressing inducible PIK3R2 shRNA, cultured with doxycycline. Cells were expanded in culture and injected subcutaneously into *scid/beige* mice. Tumours developed for several days; when they reached 50-100 mm^3, mice were doxycycline-treated (in drinking water) to induce p85beta depletion. Tumours were measured 3x weekly, and growth alone and with doxycycline treatment was compared. Graphs show individual tumour size at various times post-treatment and percent change in tumour size. Bottom, representative tumour xenograft sections of control or p85beta shRNA-treated tumours were examined by histological or active caspase-3 staining. Arrows show apoptotic cells.

Role of stress-activated protein kinase p38MAPK in human disease

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Our laboratory studies the molecular mechanisms by which p38MAP kinases, particularly p38γ and p38δ, contribute to cellular adaptation in response to inflammation in the following contexts: 1) chronic inflammation leading to tumour development (such as in colon cancer associated to colitis), 2) pathogen infection, and 3) normal inflammatory resolution occurring during tissue repair and regeneration.

Our research focuses on discovering new substrates, interacting proteins and chemical inhibitors for these kinases, and on elucidating their physiological role, using transgenic mice for distinct p38 isoforms. We also study p38MAPK as a link between chronic inflammation and cancer, and as mediator of chronic inflammatory diseases.

In the 2015-2016 period, we investigated the role of p38γ and p38δ, two less-known p38MAPK family members, in chronic inflammation and in cancer, using several mouse models. Chronic inflammation is a known risk factor for tumourigenesis. We studied the role of p38γ and p38δ in colitis-associated colon cancer and established that they are central to inflammation-induced tumour formation by regulating hematopoietic cell response to injury. We also demonstrated the pro-oncogenic role of p38γ and p38δ in the two-step DMBA/TPA carcinogenic model to induce skin tumourigenesis. We found that p38γ/p38δ-deficient mice are resistant to skin tumourigenesis. Tumour incidence and tumour burden were significantly lower in mice lacking p38γ/p38δ than in wild type mice. We also observed decreased cytokine production and proliferation in response to TPA in p38γ/p38δ-deficient mouse epithelial cells compared to wild type and single-knockout mice. Our results validate the protein kinases p38γ and p38δ as potential targets for cancer therapy.

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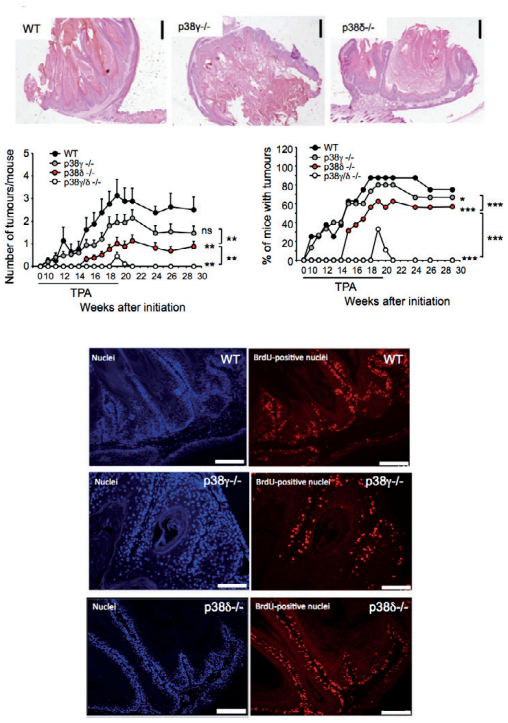

1
Top, representative images of haematoxylin-eosin staining of skin papillomas. Centre, tumour number and incidence per mouse. Bottom, representative images of total and BrdU-positive nuclei in skin papillomas. Total nuclei were stained with Hoechst33342.

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Physiopathology of chemokine receptor interactions

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SELECTED PUBLICATIONS

Vela M, Aris M, Llorente M, Garcia-Sanz JA, Kremer L. Chemokine receptor-specific antibodies in cancer immunotherapy: achievements and challenges. Front Immunol 2015; 6: 12

Bartolini F, Andres-Delgado L, Qu X, Nik S, Ramalingam N, Kremer L, Alonso MA, Gundersen GG. An mDia1-INF2 formin activation cascade facilitated by IQGAP1 regulates stable microtubules in migrating cells. Mol Biol Cell 2016; 27: 1797-1808

Roncador G, Engel P, Maestre L, Anderson AP, Cordell JL, Cragg MS, Šerbec VČ, Jones M, Lisnic VJ, Kremer L, Li D, Koch-Nolte F, Pascual N, Rodríguez-Barbosa JI, Torensma R, Turley H, Pulford K, Banham AH. The European antibody network's practical guide to finding and validating suitable antibodies for research. MAbs 2016; 8: 27-36

Chemokines are small proteins that control leukocyte trafficking and recruitment. They interact with specific G protein-coupled receptors and participate in the pathogenesis of inflammatory and infectious diseases as well as in cancer. They play a dual role in tumourigenicity. On the one hand, chemokines can help limit tumour development by increasing leukocyte migration towards the site and by inducing long-term antitumor immunity. On the other hand, they might facilitate survival, proliferation, and metastatic potential of tumour cells. Tumour expression of a chemokine receptor directs metastasis preferentially to the organs in which its ligand is secreted, suggesting chemokine receptors as promising therapeutic targets.

Our group studies how chemokines and their receptors participate in the control of tumour growth and progression, and evaluates their potential as antitumor targets.

The chemokine receptor CCR9 is expressed primarily on thymocytes and in a small subset of intraepithelial lymphocytes. Its ligand, CCL25, is expressed mainly in thymus and small intestine. CCR9 overexpression increases the migratory and invasive capacity of cancer cells, directs metastases to the small intestine and activates anti-apoptotic pathways. We generated and characterized mouse anti-human CCR9 monoclonal antibodies that reduced human T lymphoblastic cell tumours transplanted into mice by >85%. Reduction in tumour size was concomitant with an increase in the fraction of apoptotic tumour cells and necrotic areas, as well as a decrease in the fraction of proliferating cells and tumour vascularization. Our results suggest that CCR9-expressing tumours, such as those of acute and chronic T cell lineage leukaemia, might be targeted with these antibodies. Two of our antibodies have been successfully chimaerized and humanized, with affinity and specificity similar to the original antibodies. They are now being functionally characterized, using *in vitro* assays and xenogeneic animal models of human cancer.

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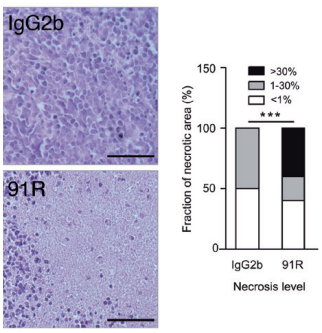
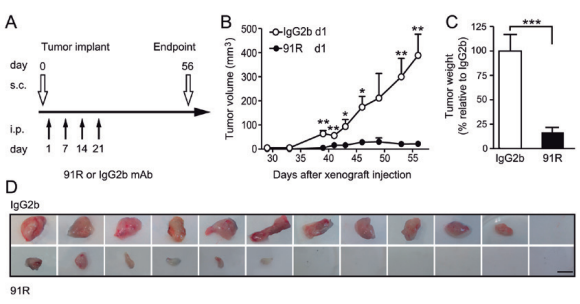


91R promotes apoptosis and necrosis and reduces cell proliferation and angiogenesis in tumour xenografts. Histological analysis of xenografted MOLT-4 tumours. Left, haematoxylin/eosin-stained sections from xenografted MOLT-4 tumours, treated with 91R or with isotype control IgG2b mAb. Right, percentage of tumours classified by necrotic stage.

2



Leukaemia xenograft growth is reduced in mice treated with 91R mAb. MOLT-4 cells were inoculated s.c. in $Rag2^{-/-}$ mice on day 0 (d0). Mice received four i.p. doses of 91R or isotype control (IgG2b) mAb and tumour growth was measured with a caliper. Mice were sacrificed, tumours were removed and weighed. (A) Antibody administration schedule. (B) Tumour growth kinetics. (C) Tumour weight (%) relative to IgG2b treatment on d56. (D) Images of tumours from mAb-treated mice at the time of sacrifice.

Signalling networks in inflammation and cancer

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SELECTED PUBLICATIONS

Gómez-Moutón C, *et al.* Filamin A interaction with the CXCR4 third intracellular loop regulates endocytosis and signaling of WT and WHIM-like receptors. Blood 2015; 125: 1116-25

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Rackov G, *et al.* p21 mediates macrophage reprogramming through regulation of p50-p50 NF-κB and IFN-β. J Clin Invest 2016; 126: 3089-103

Inflammation is a defence response of the organism to harmful internal and external stimuli. Detailed study of inflammation processes revealed a close relationship between the inflammatory reaction and the immune response. We thus expanded our initial focus on leukocyte migration to study factors and cell-cell interactions that regulate the many facets of inflammation, including differentiation of inflammatory leucocytes and changes in the endothelium that irrigates the site of injury.

In 2015-2016, we worked in three areas:

1. Characterization of the CXCR4 interaction with the actin-binding protein filamin-A

CXCR4 is a chemokine receptor involved in several human pathologies, including HIV-1 infection and tumour metastasis. Inherited mutations in CXCR4 also cause a rare immunodeficiency termed WHIM syndrome. WHIM mutations generate receptors with impaired endocytosis after agonist binding, leading to receptor hyperactivation. We demonstrated that interference with the CXCR4/filamin-A interaction restores internalization of WHIM mutant receptors, which provides a new therapeutic strategy for this rare, incurable disease.

2. Macrophage differentiation in neurological and autoimmune diseases

Innate immune cells, particularly macrophages, are major conductors of the inflammatory reaction. Depending on their polarization, macrophages can activate a "healing programme", which in cancer boosts tumour progression, or a "tissue destruction programme", as occurs in autoimmune rheumatoid arthritis (RA). We found that upregulation of microRNA (miR)-223 distinguishes macrophages from RA patients with active disease from less inflammatory macrophages from osteoarthritic patients. miR-223 targets the aryl hydrocarbon receptor pathway, increasing production of pro-inflammatory cytokines and inflammation.

3. Normalization of tumour-associated vasculature

The endothelium is a semipermeable barrier that regulates transfer of oxygen, endo- and xenobiotics, as well as tumour and immune cell diapedesis. Progressing tumours are characterized by aberrant angiogenesis but are unexpectedly highly hypoxic. The consequence is dysfunctional tumour vasculature, which reduces perfusion of tumour tissue. We showed that extracellular superoxide dismutase (SOD3) normalizes tumour vasculature, thus increasing delivery of chemotherapeutic drugs and enhancing tumour immunity.

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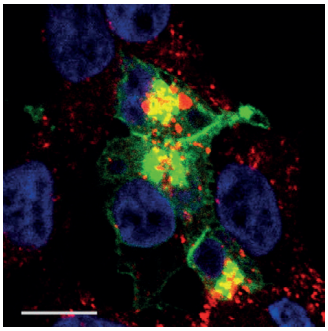
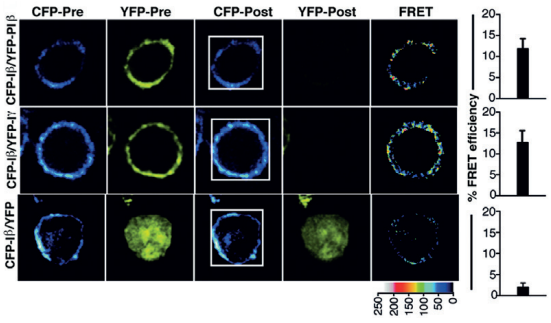


1 Agonist-induced internalization of WHIM CXCR4 mutant receptors that have lost their interaction with filamin A (in collaboration with T. Fischer, CNB-CSIC).

2



2 Dimerization of the γ and β isoforms of Type I phosphatidylinositol-4-phosphate-5-kinase as detected by FRET (in collaboration with M. Mellado's group, CNB-CSIC).

Stem cells and immunity

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SELECTED PUBLICATIONS

Villares R, Gutiérrez J, Fütterer A, Trachana V, Gutiérrez del Burgo F, Martínez-A C. Dido mutations trigger perinatal death, brain abnormalities and behavioural alterations. Proc Natl Acad Sci USA 2015; 112: 4803-8

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Berzoti-Coelho MG, Ferreira AF, de Souza Nunes N, Pinto MT, Júnior MC, Simões BP, Martínez-A C, Souto EX, Panepucci RA, Covas DT, Kashima S, Castro FA. The expression of Death Inducer-Obliterator (DIDO) variants in Myeloproliferative Neoplasms. Blood Cells Mol Dis 2016; 59: 25-30

Stem cells have the capacity for prolonged self-renewal in an undifferentiated state and for differentiation into several cell types. We identified the death inducer obliterator (*Dido*) locus as a stemness marker involved in embryonic differentiation. The Dido locus encodes three isoforms that share an N-terminal region with a nuclear localization signal (NLS), and a plant homeodomain (PHD); the latter reads chromatin state by binding trimethylated lysine of histone H3. We analysed the *Dido* PHD domain as a member of a larger family, usually proteins involved in recombination (RAG2), growth inhibition (ING), or nuclear receptors (NSD1). Mutations that affect some PHD proteins are involved in pathologies including immunological disorders, cancer and neurological diseases. Attempts to delete the entire Dido locus indicate that the gene is essential. Whereas C terminal truncation of *Dido* is lethal at embryonic day 7.5-8, its N-terminal truncation provokes aneuploidy, centrosome amplification, and cell cycle alterations, leading to altered brain development and increased incidence of hematological myeloid neoplasms. *Dido* alterations in humans are also associated with myelodysplastic syndrome (Berzoti-Coelho *et al.,* 2016) and some solid tumours. In addition to lysine trimethylation, which controls interphase gene expression, we studied the PHD domain response to histone phosphorylation in cell cycle progression (Figure 1). After cell entry into mitosis, histone phosphorylation interferes with PHD domain binding, effectively suppressing gene transcription and preparing the chromatin for subsequent chromosome condensation.

We identified anatomical and functional abnormalities of brain and craniofacial development, consistent with the known roles of primary cilia in brain patterning, hydrocephalus, and cleft palate. *Dido* N-terminal mutant mice that reached adulthood showed reduced life expectancy, brain malformations including hippocampus hypoplasia and agenesis of corpus callosum, and neuromuscular and behavioral alterations. These alterations, and the role of *Dido* in histone deacetylase 6 delivery to the primary cilium, suggest a model for the study of ciliopathies and provide information for assessing diagnosis and therapy of genetic disorders linked to deregulation of primary cilia (Figure 2).

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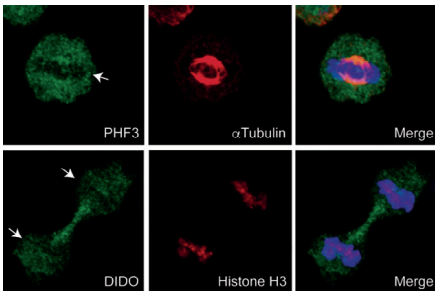

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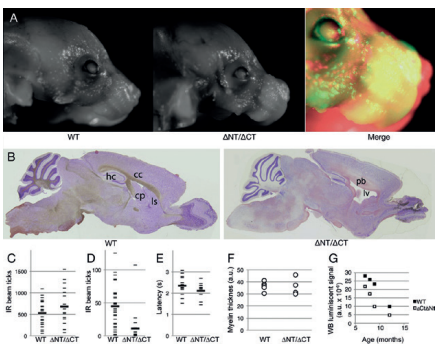
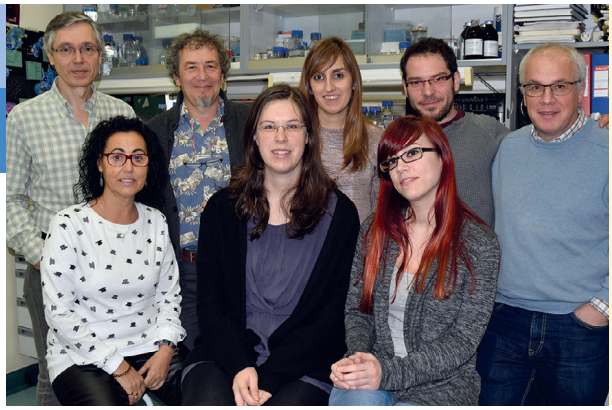


1

Epigenetic reader proteins are excluded from chromatin in mitosis. Evaluation of several PHD domain-containing proteins in dividing cells showed their exclusion from condensed chromosomes, which correlates with phosphorylation of histone residues that overlap with the PHD domain target. Mitotic expulsion of PHD proteins might have a functional role in generating a chromatin template without transcription complexes and accessible for condensin subunits.

2

Anatomical and neural anomalies *in dido* mutant mice. (A) Craniofacial defects in *dido*$^{\Delta NT/\Delta CT}$ neonates. Representative images of both genotypes, with a false-colour merged image for comparison. (B) Sagittal sections of brain from a representative *dido*$^{\Delta NT/\Delta CT}$ mouse. (C) Horizontal and (D) vertical spontaneous locomotor activity in *dido*$^{\Delta NT/\Delta CT}$ mice. (E) Plantar sensitivity to thermal stimulus in adult *dido*$^{\Delta NT/\Delta CT}$ mice. (F) Toluidine-stained myelin in cross-sections of left and right sciatic nerves from adult *dido*$^{\Delta NT/\Delta CT}$ mice and WT littermates. (G) Peripheral nerve γ-tubulin deacetylation in *dido*$^{\Delta NT/\Delta CT}$ and WT mice. (from Villares et al., 2015).



Chemokine receptors: new targets for therapeutic intervention

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SELECTED PUBLICATIONS

Cascio G, Martín-Cófreces NB, Rodríguez-Frade JM, López-Cotarelo P, Criado G, Pablos JL, Rodríguez-Fernández JL, Sánchez-Madrid F, Mellado M. CXCL12 regulates through JAK1 and 2 formation of productive immunological synapses. J Immunol 2015; 194: 5509-5519

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A broad array of biological responses, including cell polarization, movement, immune and inflammatory responses, as well as prevention of HIV-1 infection, are triggered by chemokines, a family of secreted and structurally related chemoattractant proteins that bind to class A-specific seven-transmembrane receptors linked to G proteins. Chemokines and their receptors should not be considered isolated entities, as they act in complex networks. Chemokines bind as oligomers, or oligomerize, after binding to glycosaminoglycans on endothelial cells, and are then presented to their receptors on target cells, facilitating the generation of chemoattractant gradients. The chemokine receptors form homodimers, heterodimers and oligomers at the cell surface. These structures are dynamic and are regulated by receptor expression and ligand levels. This complex scenario should be considered when analysing chemokine biology and the ability of their antagonists to act *in vivo*. Strategies based on blocking or stabilizing ligand and receptor dimers could be alternative approaches that might have broad therapeutic potential.

Our group also analyses the biological consequences of chemokine receptor activation. In the past two years, we determined that although the T cell receptor is essential for immunological synapse (IS) organization, the chemokines also participate. CXCL12-mediated signalling contributes to correct IS organization and therefore impinges on T cell activation. CXCR4 downregulation or blockade on T cells caused defective actin polymerization at the contact site with antigen-presenting cells (APC), altered microtubule-organizing centre polarization and IS structure, and also reduced the duration of T cell/APC contact. T cell activation was thus inhibited, as shown by reduced expression of CD25 and CD69 markers and diminished IL 2 mRNA levels. These results indicate that, through Gi and JAK1 and 2 kinase activation, CXCL12 signalling cooperates to build the IS and to maintain adhesive contacts between APC and T cells, which is a requisite for continuous TCR signalling.

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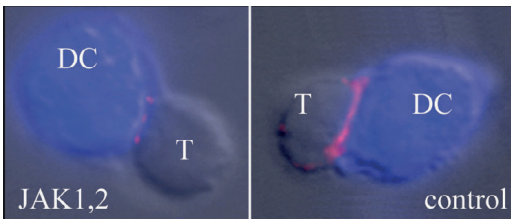

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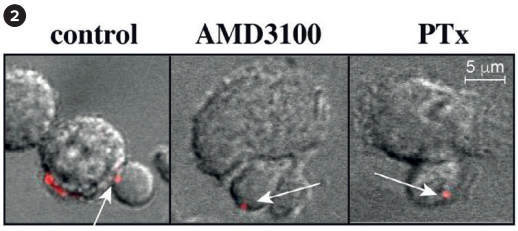

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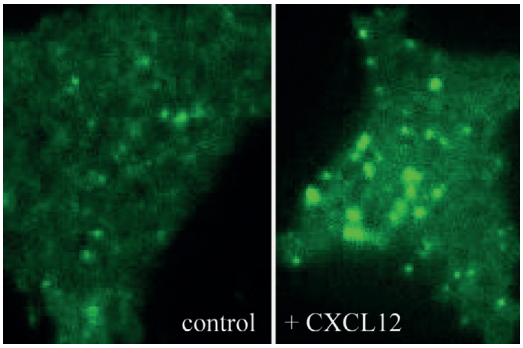

1 Detection of phosphotyrosine at an immunological synapse between JAK1,2 knock-out and wild type T cells

2 Effects of CXCR4 blockade in the localization of the microtubule-organizing centre (red) at the T/DC contact zone

3 TIRF images showing the effect of CXCL12 on CXCR4 clustering

Diacylglycerol kinases in the control of immune response and cancer progression

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Diacylglycerol kinases as negative regulators of T cell responses. DGKα and ζ limit the activation of the PLCγ/Ras/ERK axis, providing a critical checkpoint to inhibit T cell responses. High expression of these isoforms leads T lymphocytes to a hyporesponsive state, like that caused by tumours. In our laboratory, we study the mechanisms that regulate the expression and activation of these two isoforms. We also seek to determine their differential contribution to cytotoxic and regulatory T cell populations.

Diacylglycerol kinase alpha contributes to malignant transformation. DGKα is expressed abundantly in T lymphocytes, whereas it is low or even absent in other healthy cells such as melanocytes, hepatocytes or neurons. We and others have shown that DGKα expression increases in highly transformed tumours, where it contributes to the acquisition of invasive, metastatic traits. Using xenografted tumours in nude and immune competent mice, as well as 3D cultures, our group analyses the contribution of DGKα to metastasis.

Membrane traffic in the control of polarized responses. We identified sorting nexin 27 (SNX27) as a DGKζ partner in T lymphocytes. SNX27 mediates transport from endosomes to the plasma membrane of proteins that bear a specific PDZ-binding motif. In the brain, SNX27 controls receptor recycling, and its defective expression correlates with pathological conditions such as Alzheimer disease or Down's syndrome. We studied SNX27 functions using proteomic approaches and identified additional partners of this protein during immune synapse formation. Neurological and immune synapses, and tumour invadopodia are highly polarized systems that share fundamental structures necessary for correct rearrangement of actin, microtubules, adhesion molecules and lipids that facilitate vesicle trafficking. Our findings will not only help to comprehend the relevance of lipid control for T cell responses, but also allow us to understand the mechanism that links membrane traffic with neuron functions and/or tumour invasion.

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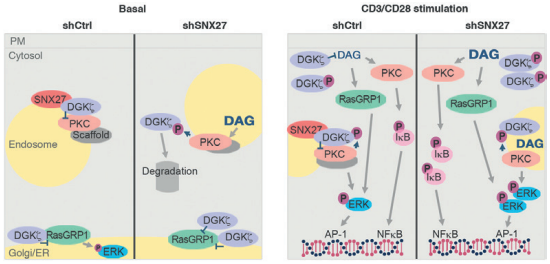

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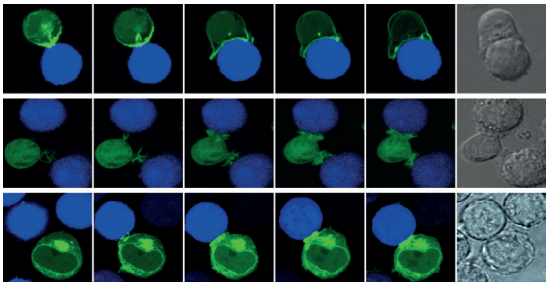

1

Model for a SNX27 role as a scaffold for lipid signalling in T cells. SNX27 is an endosomal PDZ scaffold for DGKζ-mediated control of DAG effectors activation. SNX27 allows DGKζ/PKCα reciprocal regulation; SNX27 silencing thus leads to PKC hyperactivation and subsequent DGKζ-MARCKS domain phosphorylation and degradation. SNX27 silencing in stimulated T cells (right) leads to hyperactivation of PKC and RasGRP1/ERK pathways; in contrast, SNX27 silencing in basal conditions (left) prevents DGKζ-mediated PKC modulation while it promotes DGKζ stabilization in the RasGRP1 signalling complex.

2

Rapid redistribution of DAG sensors during immune synapse formation. Jurkat T cells were transfected with tandem or individual GFP-fused PKCθ C1 domains. After 24 h, cells were plated in poly-DL-lysine-coated chambers, then mounted on a heated microscope stage. SEE-loaded Raji B cells (blue) were added to the chambers for conjugate formation and images acquired by time-lapse microscopy. The figure shows representative frames from time 0 to the end of the experiment. Right column, DIC images of T cell/APC conjugates at the end of the experiment.

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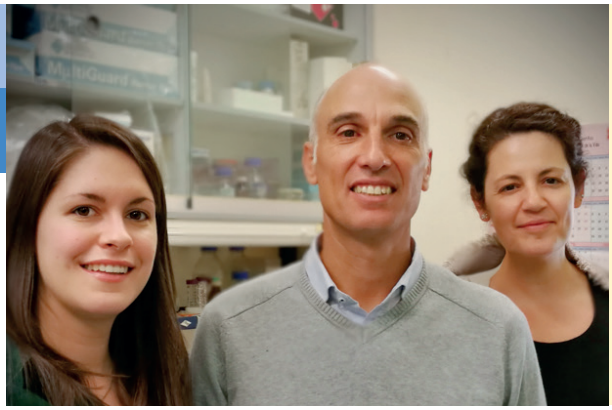

Transcriptional control of B lymphocyte differentiation

GROUP LEADER

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In Europe, 1.7 million people die each year due to cancer. It is estimated that up to 50% of all human cancers show constitutively enhanced expression of proto-oncogenes of the Myc family. The Myc proteins, members of a basic region/helix-loop-helix/leucine zipper (bHLHZip) transcription factor family (N-, L- and c-Myc), are implicated in numerous biological functions such as regulation of cell cycling, differentiation and apoptosis. Myc proto-oncogenes are deregulated by different mechanisms in one of the three myc genes or by deregulation of signalling pathways that control their expression. Many reports show that strategies that interfere with deregulated myc function will have a substantial therapeutic impact on a wide range of aggressive tumours. To activate or repress target genes, Myc proteins bind to conserved DNA sequences on gene regulatory regions; to achieve this, Myc proteins must form heterodimers with their obligate partner, Max. The majority of the scientific literature assumes that Myc function relies entirely on its capacity to form heterodimers with Max, although there are no definitive data regarding Myc/Max association. Some reports suggest that c-Myc can carry out some functions in the absence of Max in cell lines. Our group is interested in studying Myc/Max function in physiological and pathological scenarios *in vivo*, such as B lymphocyte differentiation and Myc-induced B lymphomas, respectively. We aim to characterize the role of Max in the generation and/or the maintenance of these tumours, and to identify Max-independent Myc targets as potential therapeutic tools.

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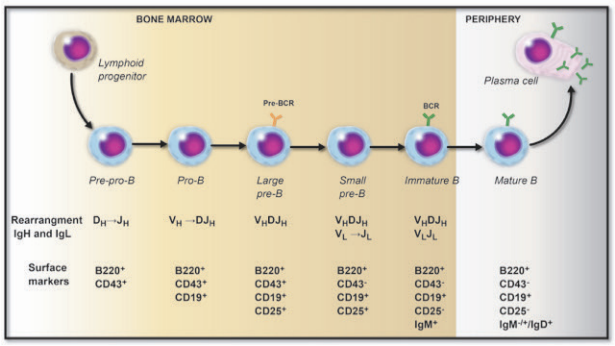

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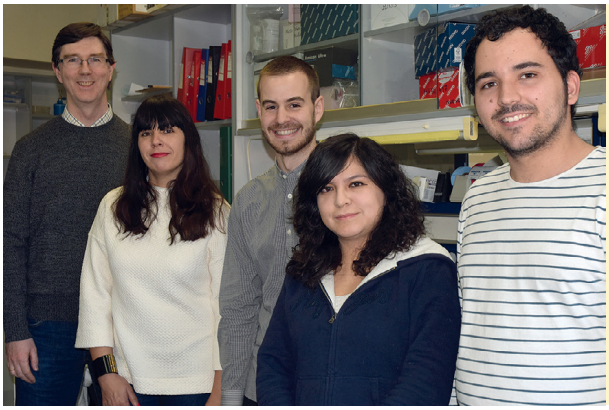
B lymphocyte differentiation in the mouse bone marrow. Scheme showing the different cell stages showing V(D)J rearrangements and some relevant surface markers.



Receptor ligand interactions in immune responses to cancer and viruses

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SELECTED PUBLICATIONS

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Reyburn HT, Esteso G, Ashiru O, Vales-Gomez M. Viral strategies to modulate NKG2D-ligand expression in Human Cytomegalovirus infection. New Horiz Transl Med 2015; 2: 159-166

Valés-Gómez M, Esteso G, Aydogmus C, Blázquez-Moreno A, Marín AV, Briones AC, Garcillán B, García-Cuesta EM, López Cobo S, Haskologlu S, Moraru M, Cipe F, Dobbs K, Dogu F, Parolini S, Notarangelo LD, Vilches C, Recio MJ, Regueiro JR, Ikinciogullari A, Reyburn HT. Natural killer cell hyporesponsiveness and impaired development in a CD247-deficient patient. J Allergy Clin Immunol 2016; 137: 942-5

Romera-Cárdenas G, Thomas LM, Lopez-Cobo S, García-Cuesta EM, Long EO, Reyburn HT. Ionomycin Treatment Renders NK Cells Hyporesponsive. PLoS One 2016; 11: e0150998

Our research is focused on two main areas, understanding the roles of natural killer (NK) cells in response to virus infections, and study of NK cell function in patients suffering primary immunodeficiencies (whose major clinical problems are virus infections). We are also interested in the mechanisms by which viral pathogens try to evade NK cell immune surveillance.

In these studies, we aim to provide new tools to aid in the diagnosis and therapy of these diseases as well as to generate new insights into the biology and function of the immune system that will be relevant to the fields of vaccination, immune responses to cancer and autoimmunity.

For example, our analyses of immunodeficient patients have shown that NK cells in these patients are often immature both phenotypically and functionally. In some cases, this appears to be a direct effect of the immunodeficiency causing mutation; however, in other cases, our observations raise the exciting hypothesis that disruption of the normal crosstalk between immune effectors can impact negatively on the differentiation and function of the remaining cells of the immune system, and so contribute to the spectrum of pathology in these patients. Testing this hypothesis to gain a full understanding of the pathophysiological significance of these immune system changes requires high-resolution analysis of these altered cell populations, where possible at the single cell level, using new, highly multiplexed technologies that allow detailed phenotypic, functional and molecular analyses of single immune cells.

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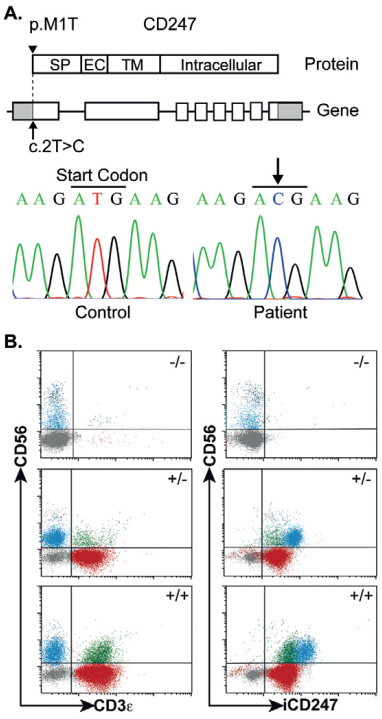

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Genetic and phenotypic characterization of CD247 deficiency. (A) The index patient inherited CD247 mutation affecting the start codon is shown within the CD247 protein and gene structure, as well as in the comparative chromatograms. (B) White blood cells from the CD247 deficient patient (-/-), a relative heterozygous for the CD247 initiation codon mutant (+/-) and a family member homozygous for non-mutant CD247 sequence (+/+) were stained with directly labelled mAbs specific for CD56.

T cell signalling in autoimmune diseases and cancer

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SELECTED PUBLICATIONS

González-Martín A, Adams BD, Lai M, Shepherd J, Salvador-Bernaldez M, Salvador JM, Lu J, Nemazee D, Xiao C. The microRNA miR-148a functions as a critical regulator of B cell tolerance and autoimmunity. Nat Immunol 2016; 17: 433-440

The main goals of our research are a) to study the *in vivo* function of the Gadd45 family and p38 in development, suppression of autoimmunity and cancer, and b) to analyse the underlying molecular mechanisms to identify therapeutic targets for these diseases.

To address these goals, we generated and characterized several lines of Gadd45 and p38 MAPK knockout mice. Gadd45a-null mice develop a lupus-like syndrome, characterized by high autoantibody titres, lymphoproliferation, autoimmune glomerulonephritis and premature death. We previously found that Gadd45a acts as an autoimmune disease suppressor gene by blocking p38 activation in T cells. In addition, Gadd45a functions as a dose-sensitive regulator of B cell tolerance. Lack of Gadd45a in mice allows escape of autoreactive B cells from the central tolerance checkpoint. Dysregulation of this molecular mechanism might contribute to the development of autoimmune disease in Gadd45a-null mice. The proposed model is that elevated miR-148a expression impairs B cell tolerance by promoting the survival of immature B cells by suppressing Gadd45a expression. Furthermore, increased miR-148a expression facilitated the development of lethal autoimmune disease in a lupus mouse model.

To dissect p38α and p38β functions in T cells, we characterized mice that lack p38α or p38β, and generated double-knockout mice. Since lack of p38α in mice causes embryonic lethality, we used a conditional knockout mouse model (Mapk14Δ/Δ) to analyse p38α function. Our results indicate that Mapk14Δ/Δ mice provide a useful model for the analysis of p38α deficiency in CD4+ T cells. We found an unpredicted function for p38 MAPK in the control of CD4+ T cell proliferation following TCR activation. We also examined the role of p38α and p38β in IFNγ production, and found that p38α and p38β control T cell receptor-induced IFNγ and TNFα production, but only p38α modulates cytokine-induced IFNγ production.

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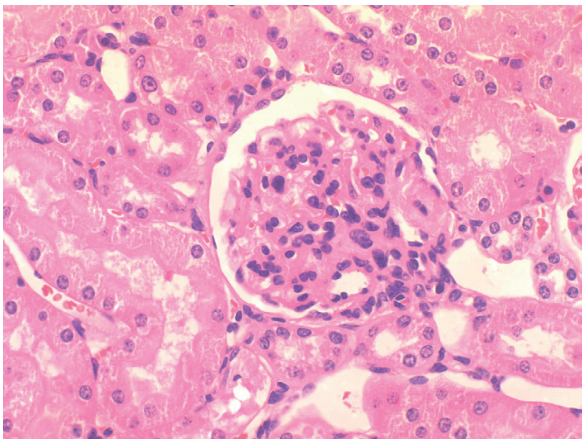

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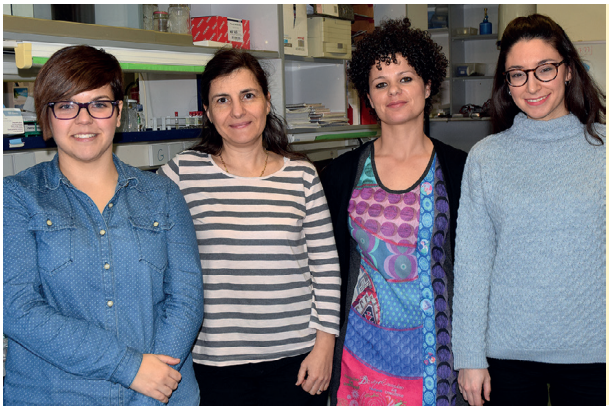
Image showing the spontaneous development of autoimmune glomerulonephritis in Gadd45$^{-/-}$ mice



Tumour immune activation and evasion

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Carmen Campos Silva

Our group is interested in the modulation of natural killer (NK) cell activation in the context of cancer. For this purpose, we study *in vitro* models and *ex-vivo* patient samples mainly from two types of human tumours, bladder cancer and melanoma. We use a range of biochemical, molecular and immunological techniques to understand the differentiation and proliferation events that occur in the NK cell compartment in the context of cancer and the changes provoked by therapies. For example, in the case of bladder cancer, we follow the treatment with BCG (Bacille Calmette-Guérin), whereas in melanoma, we study BRAF inhibitors. Our main interest consists of describing the detailed phenotype of NK cells with anti-tumour capacities and the factors required for their differentiation. In parallel, we study immune-modulating molecules such as the tumour-secreted ligands for the activating receptor NKG2D. These ligands can be soluble or released as part of extracellular vesicles such as exosomes.

We are also interested in developing exosome-based tools for immune modulation and technologies that will permit easier detection of exosomal biomarkers in cancer. To pursue this goal, we have started to establish collaborations with clinicians to have access to samples, with researchers and technological partners to develop the tools, and we also form part of a new Spanish network for research in extracellular vesicles.

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García-Cuesta EM, López-Cobo S, Álvarez-Maestro M, Esteso G, Romera-Cárdenas G, Rey M, Cassady-Cain RL, Linares A, Valés-Gómez A, Reyburn HT, Martínez-Piñeiro L, Valés-Gómez M. NKG2D is a key receptor for recognition of bladder cancer cells by IL-2-activated NK cells and BCG promotes NK cell activation. Front Immunol 2015; 6: 284

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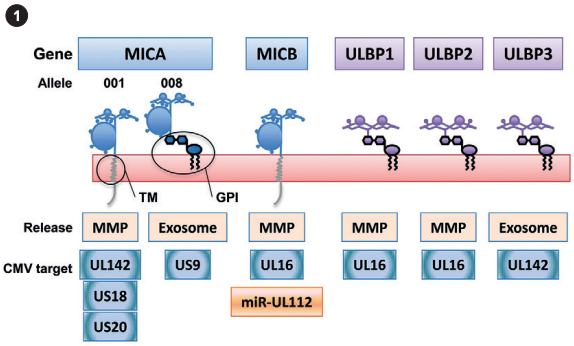
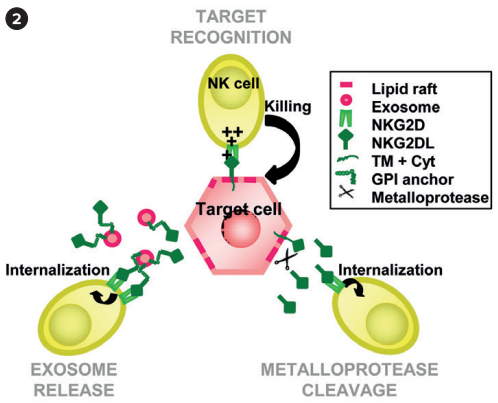


1 NKG2D ligands belong to two genetic families with numerous different biochemical and cell biology properties. These families, MICA/B (major histocompatibility complex class I-related chain) and ULBP (UL16-binding proteins) encode proteins with distinct membrane attachments [transmembrane (TM) and glycosyl-phosphatidylinositol (GPI)] and distinct mechanisms for release to the extracellular milieu (exosomes vs. metalloprotease cleavage). Not all NKG2D ligands in a family share the same biochemical characteristics; they are also recognized differentially by viral proteins and miRNAs that interfere with the immune response. Here, the human cytomegalovirus evasion molecules that target NKG2D ligands (proteins in blue, miRNA in orange) are depicted (from Valés-Gómez, 2015).



2 NKG2D ligand biology. NKG2D ligands (NKG2DL) are recognized by NKG2D on the target cell surface, leading to activation of degranulation machinery by the effector cell and target cell death. NKG2DL can be recruited to detergent-resistant membranes (lipid rafts) and be cleaved by metalloproteases, resulting a soluble ligand, or can be included in exosomes, resulting in multimeric membrane-bound ligands. Interaction with soluble NKG2DL, as cleaved proteins or as part of exosomes, can block and downmodulate the receptor and lead to immune evasion (from López-Cobo *et al.*, 2016).