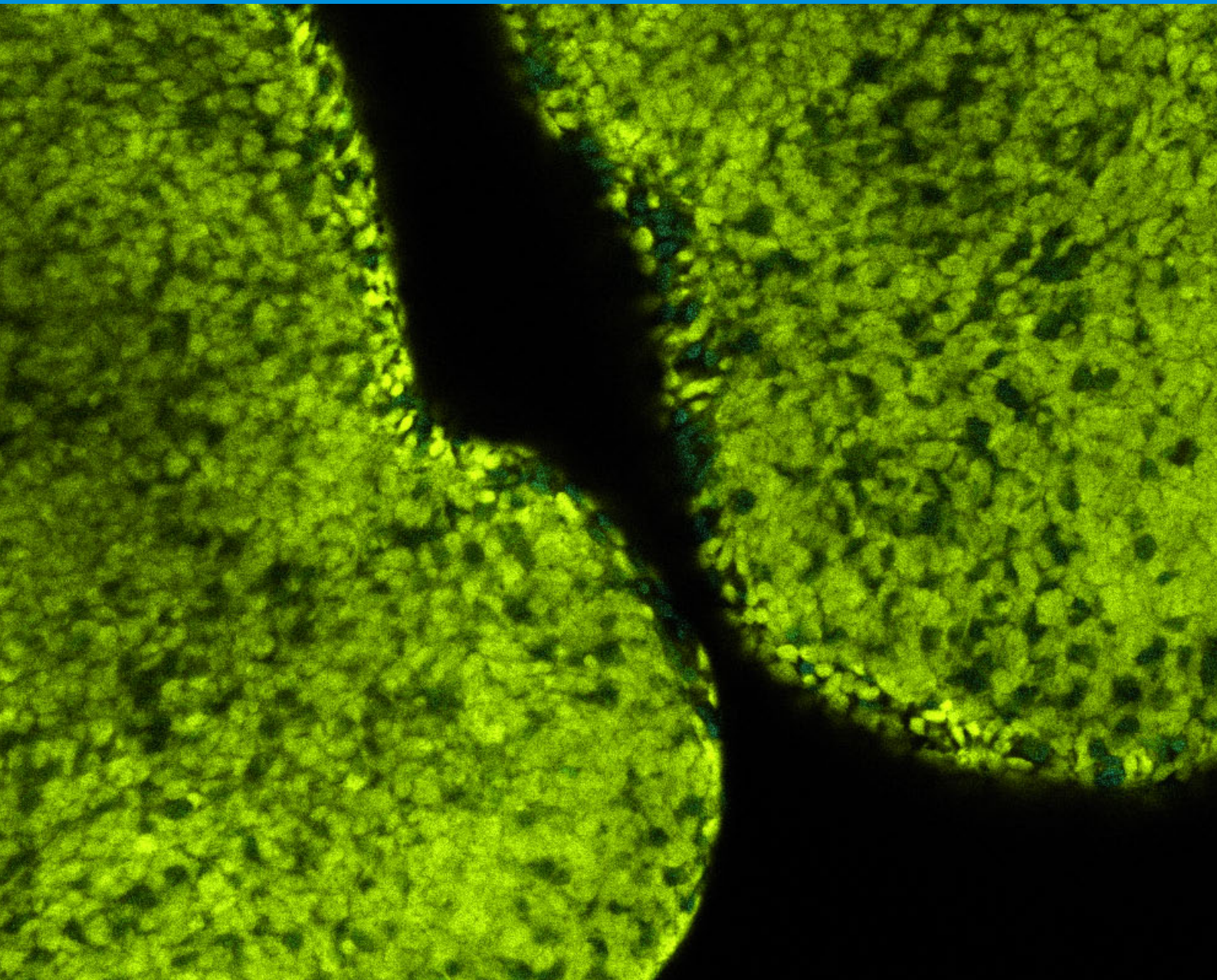
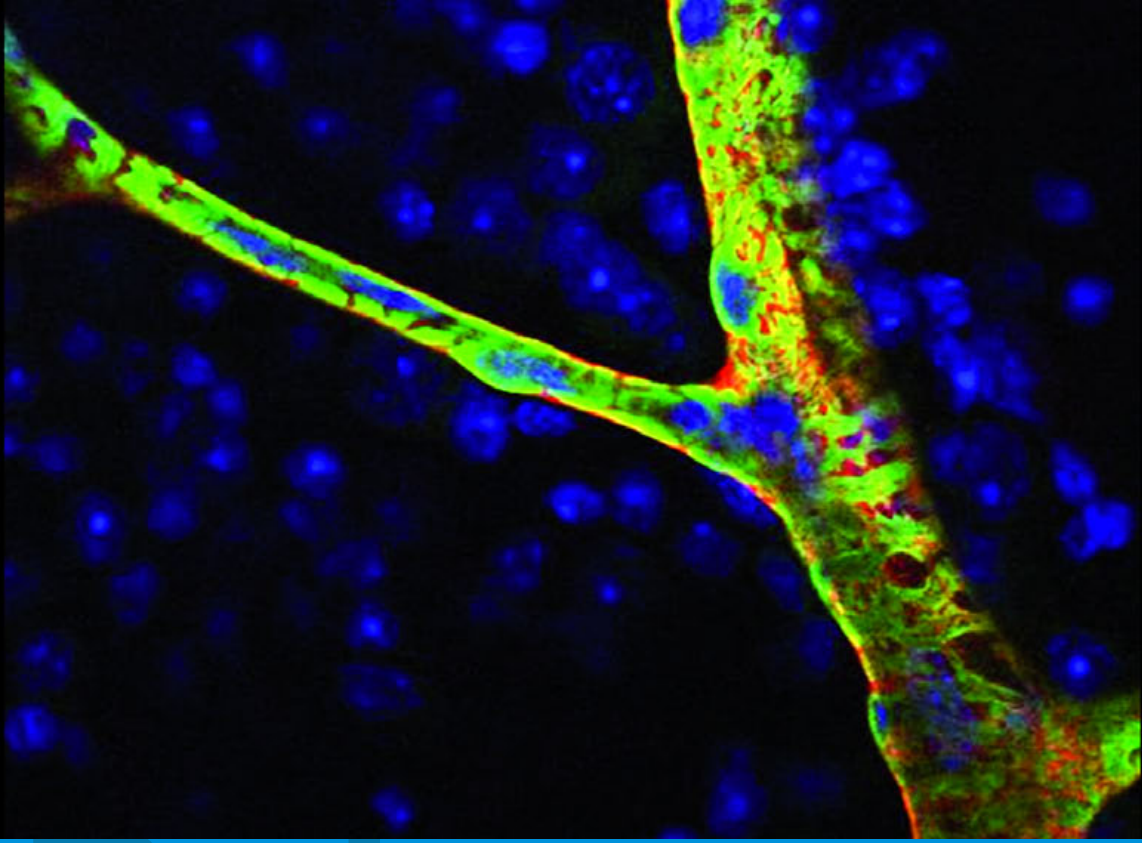


Basic Research Departments

3

Vascular Biology and Inflammation





Basic Research Departments

3 Vascular Biology and Inflammation

The Department of Vascular Biology and Inflammation investigates interactions between the cells of the vascular system. Specific research lines address signaling by adhesion receptors and inflammatory mediators, physiological and pathological angiogenesis, and vascular wall remodeling. Groups within the department use a range of animal, tissue, cellular and molecular models to investigate normal vascular function and the key steps in the vascular alterations that underlie cardiovascular diseases.

DEPARTMENT DIRECTOR: *Juan Miguel Redondo*

DEPARTMENT MANAGER: *Antonio Jesús Quesada*

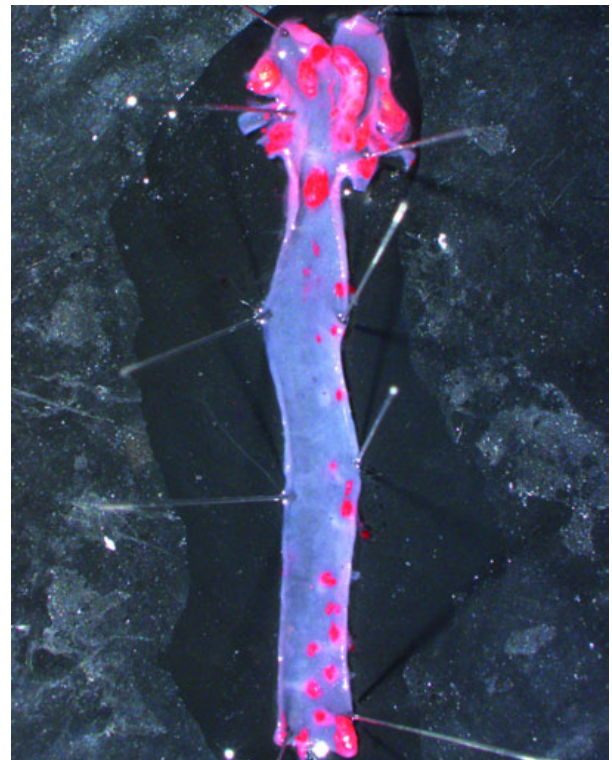
TECHNICIANS: *Andrea Quintana
Juan José Lazcano*

ADMINISTRATIVE SUPPORT: *Almudena Fernández
María Jesús de la Calle*

*Gene regulation in cardiovascular
and inflammatory diseases***Head of Laboratory:** *Juan Miguel Redondo***Postdoctoral Researchers:** *Pablo Gómez-del Arco
Sara Martínez-Martínez
Aránzazu Alfranca
Miriam Zeini
Vanesa Esteban***Predocctoral Researchers:** *Katia Urso
Amelia Escolano
Nerea Méndez
Noelia Lozano***Masters Students:** *Jorge Oller
María del Mar Torres***Technicians:** *Dolores López Maderuelo
Felipe Were
Raquel Sánchez
Ana Guio
Gema Benito
Beatriz Carolina Ornés***RESEARCH INTEREST**

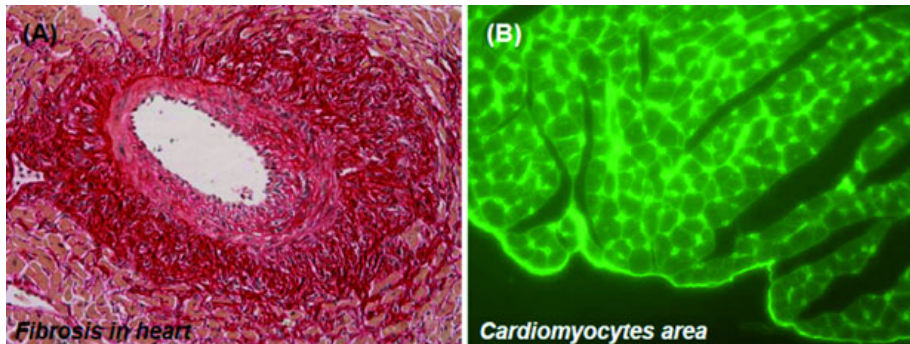
The calcium-calcineurin-NFAT (CN-NFAT) pathway regulates heart valve morphogenesis, pancreatic beta-cell function and the development of the immune, vascular and nervous systems, and is implicated in many related pathological processes. We study the regulation and function of CN-NFAT signaling in lymphocyte activation, angiogenesis and cardiac hypertrophy. Much of our work relates to molecular interactions of the phosphatase calcineurin with NFAT transcription factors and other substrates and regulators. This work has identified sequence motifs important for these interactions and sheds light on the mechanism of immunosuppressive drugs.

Our work on angiogenesis addresses the regulation of NFAT in endothelial cells by VEGF and the profile and actions of prostanoids released by activated endothelium. We use retinopathy of prematurity (ROP) as a model of the mechanisms of neovessel formation in ischemic retinopathies, and are using lentiviral vectors to identify potential therapeutic targets. We are also analyzing the role of CN in different mouse models of chronic inflammatory diseases, as well as the gene expression program triggered by angiotensin II (Ang-II) in cardiomyocytes and vascular smooth muscle and the role of CN-NFAT signaling in these processes. This work is being conducted through the use of mouse models of vascular remodeling, including inward remodeling (restenosis) and outward remodeling (aneurysm), and is shedding light on the signaling pathways involved in these diseases.

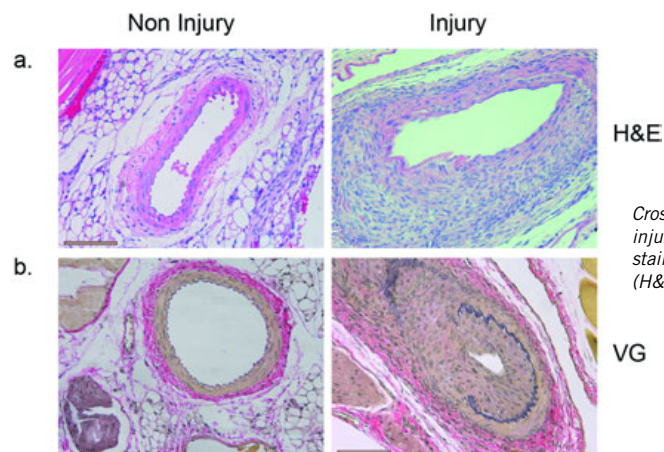


Oil red staining of atheroma plaques in the aorta of a mouse fed a high-cholesterol diet.

3 Vascular Biology and Inflammation



Ang-II causes fibrosis and hypertrophy in the heart. The images show heart sections from a mouse infused with Ang II during 20 days. Left: Picrosirius red staining showing expanded fibrous tissue surrounding heart vessels. Right: An area of hypertrophic cardiac tissue, revealed by staining for cardiomyocytes with fluorescently-labeled wheat germ agglutinin.



H&E

Cross-sections of uninjured and injured mouse femoral arteries stained with hematoxylin-eosin (H&E) and Van Gieson's stain (VG).

VG



MAJOR GRANTS

- Ministerio de Ciencia e Innovación (SAF2009-10708)
- Instituto de Salud Carlos III. Red RECAVA (RD06/0014/0005)
- Comunidad de Madrid (S-BIO-0194-2006)
- Fundación Genoma España
- Fundació La Marató TV3 (081731)



SELECTED PUBLICATIONS

Bonzon-Kulichenko E, Pérez-Hernández D, Núñez E, Martínez-Acedo P, Navarro P, Trevisan-Herraz M, Ramos Mdel C, Sierra S, Martínez-Martínez S, Ruiz-Meana M, Miró-Casas E, García-Dorado D, Redondo JM, Burgos JS, Vázquez J. **A robust method for quantitative high-throughput analysis of proteomes by ^{18}O labeling.** *Mol Cell Proteomics* (accepted)

Gómez-del Arco P, Kashiwagi M, Jackson AF, Naito T, Zhang J, Liu F, Kee B, Vooijs M, Radtke F, Redondo JM, Georgopoulos K. **Alternative promoter usage at the Notch1 locus supports ligand-independent signaling in T cell development and leukemogenesis.** *Immunity* (2010) 33: 685-98

Rodríguez A, Roy J, Martínez-Martínez S, López-Maderuelo MD, Niño-Moreno P, Ortí L, Pantoja D, Pineda-Lucena A, Cyert M, Redondo JM. **A conserved docking surface on calcineurin mediates interaction with substrates and immunosuppressants.** *Mol Cell* (2009) 33: 616-26 (Highlighted as "must read paper" in Faculty of 1.000)

Martínez-Martínez S, Genescà L, Rodríguez A, Salichs E, Raya A, Were F, López-Maderuelo MD, Redondo JM* and S. de la Luna* **The RCAN carboxyl-end mediates calcineurin docking-dependent inhibition via a site that dictates binding to substrates and regulators.** *Proc Natl Acad Sci USA* (2009) 14: 6117-22

*Co-corresponding author

Salvado MD, Alfranca A, Escolano A, Haeggström J, Redondo JM. **COX-2 limits prostanoid production in activated endothelial cells and is a source of PGH_2 for transcellular metabolism to PGE_2 by tumor cells.** *Arterioscler Thromb Vasc Biol* (2009) 29: 1131-7

Integrin signaling



Head of Laboratory: Miguel Ángel del Pozo

Research Scientist: Asier Echarri

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Ana Cerezo
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Predoctoral Researchers: Marta C. Guadamillas
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Teresa Osteso



RESEARCH INTEREST

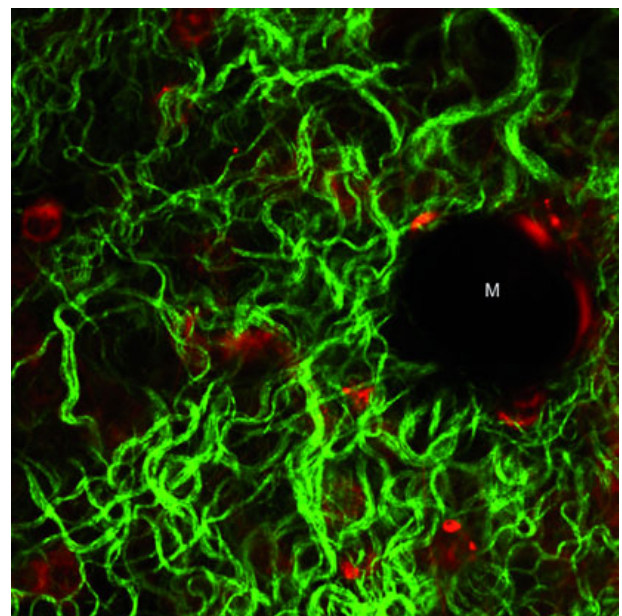
Signals are the language of life, mediating the communication essential for cells' proper behavior. Within cells, intricate networks of proteins transduce signals into the appropriate physiological response, and many diseases are caused by malfunctioning of these signal transduction networks. Our interest is in the mechanisms through which integrins, Rho/Rac GTPases and caveolin-1 (Cav1) cooperate to regulate gene expression, cell cycle progression, migration, polarization, vesicle trafficking and epithelial-mesenchymal transition (EMT), key processes in the pathogenesis of cancer and inflammatory and cardiovascular diseases.

A growing body of work supports a role for caveolae and Cav1 in mechanosensing and mechanotransduction. We have shown that Cav1 can modulate cell shape and responses via force-dependent remodeling of the 3D microenvironment.

Loss of integrin-mediated adhesion triggers an inward traffic of Cav1-rich membranes, which regulates Rac1 plasma membrane (PM) targeting and hence directs cell migration and controls cell proliferation. We have now found that Rac1 can be palmitoylated, which favors its stabilization in liquid-ordered areas of the PM, inducing actin polymerization which causes the coalescence of ordered microdomains into larger regions, thus promoting PM order. Our recent work has delineated how filamin A regulates actin-linked caveolae dynamics at the PM, and shows that Cav1-membrane inward trafficking depends on actin, microtubules (MT), dynamin2, Abl kinases, the formin mDia1, and phosphorylation of filamin A by PKC α . After de-adhesion, internalized Cav1-rich rosettes are transferred to a MT-dependent system that targets them to a Rab11-recycling endosome. In response to cell adhesion, Cav1 recycles back to the PM via a mechanism involving Abi1-Arp2/3-mediated branched actin polymerization. Cav1 will form caveolae as stress fibers are formed, but caveolae are flattened by high PM tension

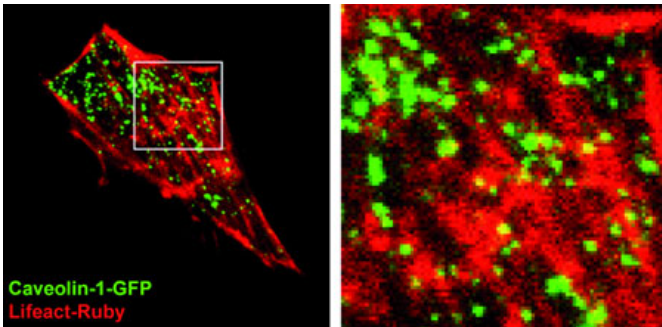
induced by excessive mDia1-actin-mediated force. To fully understand the molecular mechanisms by which integrins regulate Cav1 trafficking, we are conducting an RNAi-based high-content image analysis screen in collaboration with the Cellomics Unit.

Our work on EMT and fibrosis during chronic peritoneal inflammation has identified an inducing role for the ERK/NF- κ B/Snai1 pathway, while p38 MAPK acts as a brake.

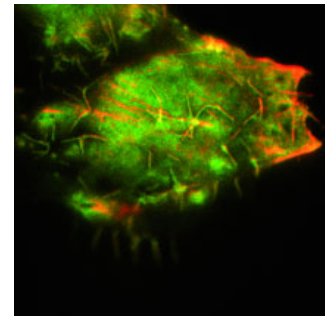


Multiphoton excitation microscopy image showing second harmonic generation (SHG, green) signal and autofluorescence (red) in intact mammary tissue from wild-type mice. The green staining reflects the degree of parallelism in collagen fibers. M=Mammary gland

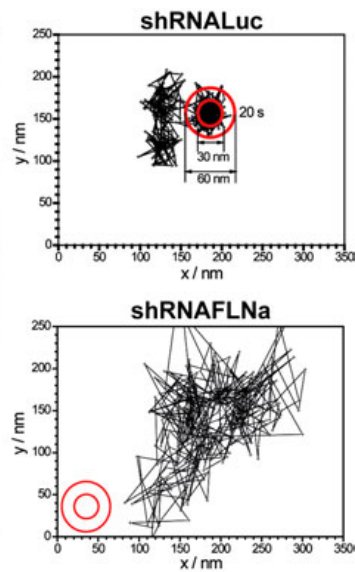
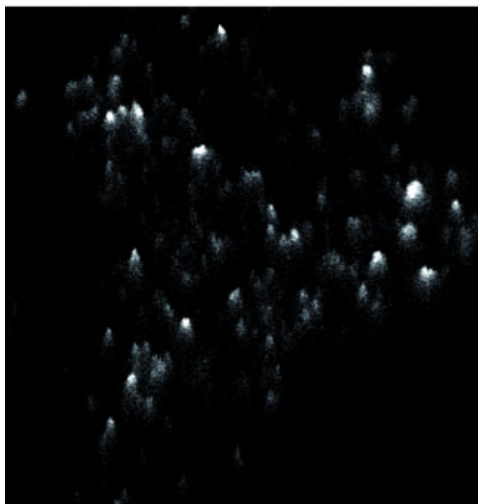
3 Vascular Biology and Inflammation



Total internal reflection fluorescence (TIRF) microscopy image at 90 nm penetration showing caveolin vesicles and actin fibers (stained with RFP-Ruby-Lifeact) in HeLa cells.



TIRF microscopy showing colocalization of GFP-tagged wild-type Rac1 (green) with the actin marker Lifeact (red) on the ventral surface of live COS7 cells.



High spatio-temporal resolution particle tracking of Cav1-GFP vesicles by TIRFm in control HeLa cells (shRNALuc) or filamin A depleted cells (shRNAFLNa). Sequential vesicle positions were recorded at 85 ms intervals and connected by straight lines. Outer circles show the threshold for an anchoring event (60 nm diameter); inner circles show the positioning accuracy (30 nm). Duration of anchoring events is indicated.



MAJOR GRANTS

- Ministerio de Ciencia e Innovación (SAF2008-02100)
- Ministerio de Ciencia e Innovación. Consolider COAT (CSD2009-00016)
- Instituto de Salud Carlos III. Red RTICC (RD06/0020/1033) (PI: Asier Echarri)
- European Science Foundation. EURYI (European Young Investigator Award, 2005-2010)



SELECTED PUBLICATIONS

Strippoli R, Benedicto I, Pérez-Lozano ML, Foronda M, Sánchez-Perales S, López-Cabrera M and del Pozo MA. **p38 maintains E-cadherin expression by modulating TAK1-NF- κ B during epithelial-to mesenchymal transition** (2010) *J Cell Science* 123: 4321-31

Gonzalo P, Guadamillas MC, Hernández-Riquer MV, Pollán A, Grande-García A, Bartolomé RA, Vasanji A, Ambrogio C, Chiarle R, Teixidó J, Ristel J, Apte SS, del Pozo MA, and Arroyo AG. **MT1-MMP is required for myeloid cell fusion via regulation of Rac1 signaling** (2010) *Dev. Cell* 18: 77-89

Cerezo A, Guadamillas MC, Goetz J, Sánchez-Perales S, Klein E, Assoian R and del Pozo MA. **Absence of caveolin-1 increases proliferation and anchorage-independent growth by a Rac-dependent, Erk-independent mechanism.** *Mol and Cell Biol* (2009) 29: 5046-59

Strippoli R, Foronda M, López-Cabrera M and del Pozo MA. **Targeting the ERK/NF- κ B/Snail1 pathway as a potential therapeutic strategy to prevent the failure of peritoneal dialysis.** *Nature Rev Cardiol* (CNIC Edition) (2009) 6: 43-8

Matrix metalloproteinases in angiogenesis and inflammation



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Rubén A. Mota

Predocctoral Researchers: María Victoria Hernández de Riquer
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Mara Martín
Vanessa Moreno

Masters Student: Cristina Clemente

Technician: Ángela Pollán

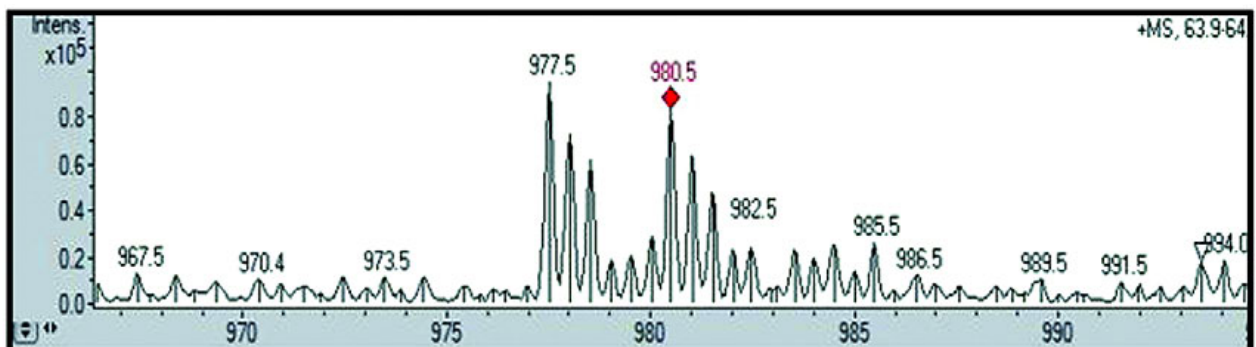


RESEARCH INTEREST

Angiogenesis in adults is often coupled to inflammation, and its deregulation might contribute to the development and progression of chronic inflammatory disease. We previously reported the contribution of the matrix metalloproteinase MT1-MMP to inflammation and angiogenesis, in particular to chemokine and nitric oxide-induced angiogenesis, monocyte transmigration, and more recently to myeloid cell fusion. This latter function requires binding of the MT1-MMP cytosolic tail to the adaptor p130Cas, thereby upregulating Rac1 membrane targeting and activity. This finding indicates that the functions of MT1-MMP in inflammation are cell context-dependent, and to explore this in more depth we have conducted proteomic analyses to identify the collection of cellular substrates (degradome) processed by MT1-MMP in endothelial cells and leukocytes. We have also applied this approach to MT4-MMP, a GPI-anchored MMP whose substrates and functions are poorly understood. Preliminary data point to specific and unexpected functions for these proteases in the interplay between inflammation and angiogenesis.

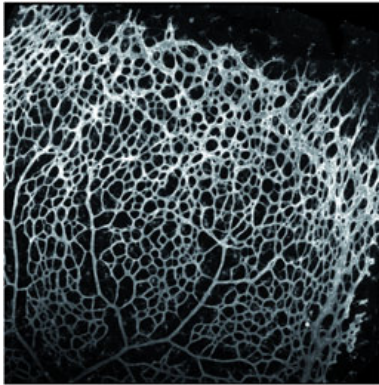
We are using the novel molecular information generated by the proteomic analysis to explore key events in inflammation-driven angiogenesis, such as the induction of endothelial tip cells and the decision between stabilization and regression of the new vasculature, and how these processes are linked to the phenotype of macrophages and other components of the inflammatory infiltrate. These functional studies are conducted in cell-based systems and in genetically modified mouse models of angiogenesis and inflammatory disorders such as atherosclerosis. We are also characterizing the role of more recently identified players in vascular integrity and angiogenesis, mainly MT-MMP substrates such as extracellular matrix metalloproteinase inducer (EMMPRIN).

Through these efforts we aim to extend our knowledge of where, when and how MT-MMPs and their substrates modulate endothelial and leukocyte behaviour during the establishment and progression of chronic inflammatory disorders.

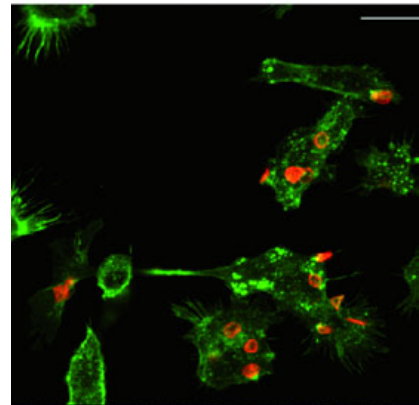
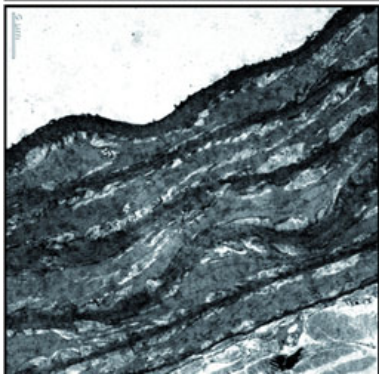


SILAC (stable isotope labeling of aminoacids in culture) is a quantitative proteomic approach that we are using to identify the degradome of specific proteases in cell types involved in inflammation and angiogenesis. The figure shows a mass spectrum obtained from an actin peptide labeled with heavy or light amino acids.

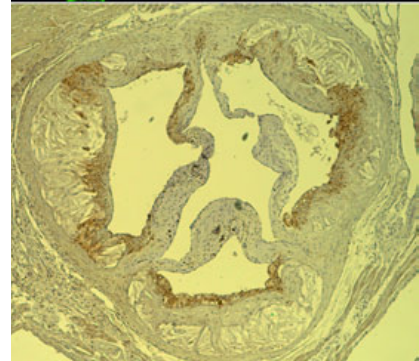
3 Vascular Biology and Inflammation



Analysis of angiogenesis and vascular homeostasis. Top: Whole-mount staining with isolectin B4 reveals active angiogenesis in mouse retinas seven days postpartum. Bottom: Electron micrograph showing the ultrastructure of the mouse aortic wall.



Macrophages and inflammation. Top: Mouse peritoneal macrophages (green) can engulf sheep red blood cells (red) by phagocytosis. Bottom: Mac-3 staining reveals the presence of macrophages in atherosclerotic plaques in LDLR^{-/-} mice fed a high fat diet.



MAJOR GRANTS

- Ministerio de Ciencia e Innovación (SAF2008-0214)
- Ministerio de Ciencia e Innovación. FIS RETICS (RECAVA; RD/06/0014/1016)
- Fundación Genoma España. MEICA Project



SELECTED PUBLICATIONS

Arroyo AG, Iruela-Arispe ML. **Extracellular matrix, inflammation, and the angiogenic response.** *Cardiovasc Res* (2010) 86: 226-35

Gonzalo P, Arroyo AG. **MT1-MMP: A novel component of the macrophage cell fusion machinery.** *Commun Integr Biol* (2010) 3: 1-4

Gonzalo P, Guadamillas MC, Hernández-Riquer MV, Pollán A, Grande-García A, Bartolomé RA, Vasani A, Ambrogio C, Chiarle R, Teixidó J, Risteli J, Apte SS, del Pozo MA, Arroyo AG. **MT1-MMP is required for myeloid cell fusion via regulation of Rac1 signaling.** *Dev Cell* (2010) 18: 77-89

Gonzalo P, Moreno V, Galvez BG and Arroyo AG. **MT1-MMP and integrins: Hand-to-hand in cell communication.** *Biofactors* (2010) 36: 248-54

Nunez V, Alameda D, Rico D, Mota R, Gonzalo P, Cedenilla M, Fischer T, Bosca L, Glass CK, Arroyo AG and Ricote M. **Retinoid X receptor alpha controls innate inflammatory responses through the up-regulation of chemokine expression.** *Proc Natl Acad Sci U S A* (2010) 107: 10626-31

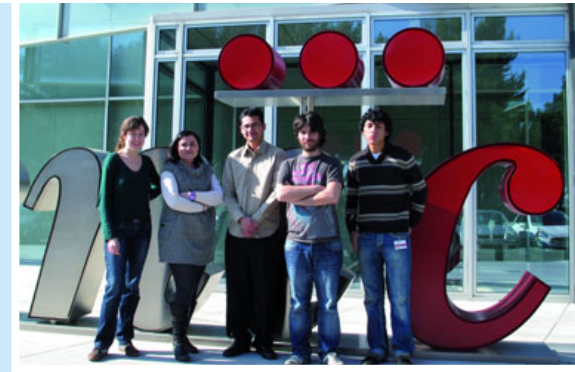
Regulatory molecules
of inflammatory processes

Head of Laboratory: Pilar Martín

Postdoctoral Researcher: José Rodríguez Cortés

Predoctoral Researchers: Aránzazu Cruz Adalia
Adela Matesanz Marín

Visiting Scientist: César Augusto Henríquez Camacho

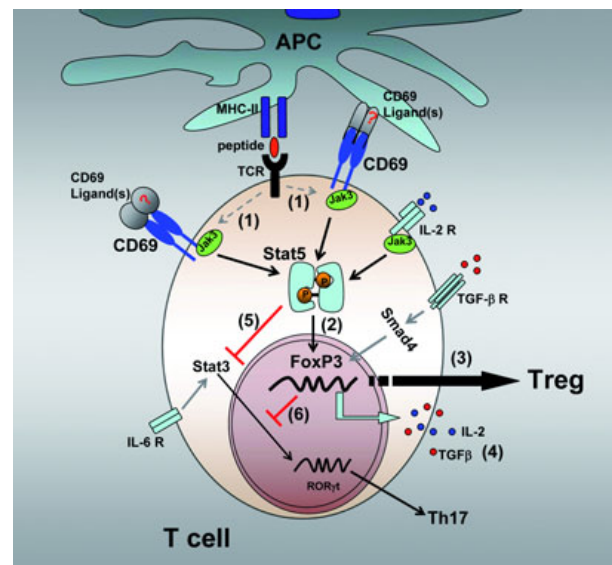


RESEARCH INTEREST

Understanding peripheral mechanisms operating in autoimmune and chronic inflammatory diseases is critical for the design and development of novel therapies against these immunological disorders. Autoimmune diseases are characterized by a breakdown in the mechanisms of tolerance to self antigens. Autoimmune diseases, which include conditions such as arthritis, asthma, contact dermatitis and myocarditis, affect millions of people worldwide, and there is no definitive treatment for their eradication. Our group seeks to identify new regulatory cells and molecules involved in the control of these diseases.

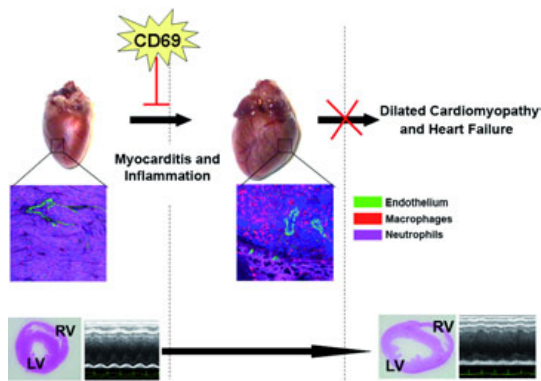
The early leukocyte activation antigen CD69 is a membrane receptor ascribed to the family of type II C-type lectins. It is rapidly induced after cell activation in all bone marrow derived cells except erythrocytes. Expression *in vivo* is restricted to positively selected thymocytes and leukocytes undergoing activation, particularly at inflammatory sites. Engagement of CD69 with monoclonal antibodies in the presence of phorbol esters induces Ca^{2+} influx that leads to the activation of ERK, induction of IL-2 and IFN- γ genes, and T cell proliferation. Our recent work shows that the cytoplasmic tail of CD69 interacts with Jak3/Stat5 proteins, which regulate the transcription of ROR γ t in human and mouse Th17 cells, thus establishing a mechanistic link between CD69 and the regulation of Th17 differentiation. The balance between Th17 cells and regulatory T cells determines the net balance between pro- and anti-inflammatory cytokines at inflammatory foci, and is thus critical for the regulation of the immune response. CD69 might also regulate the function or differentiation of regulatory T cells, thus affecting the outcome of Th17 responses indirectly. This is supported by the finding that mice lacking CD69 develop exacerbated forms of contact dermatitis, allergic asthma and autoimmune myocarditis. Our data demonstrate that CD69, through the regulation of Th17 effector responses, limits myocardial inflammation and subsequent heart failure. It is likely that a similar process occurs in humans with myocarditis and subsequent dilated

cardiomyopathy. These findings reveal the involvement of a novel molecular actor in the immunopathogenesis of myocarditis, which could be a potential therapeutic target.

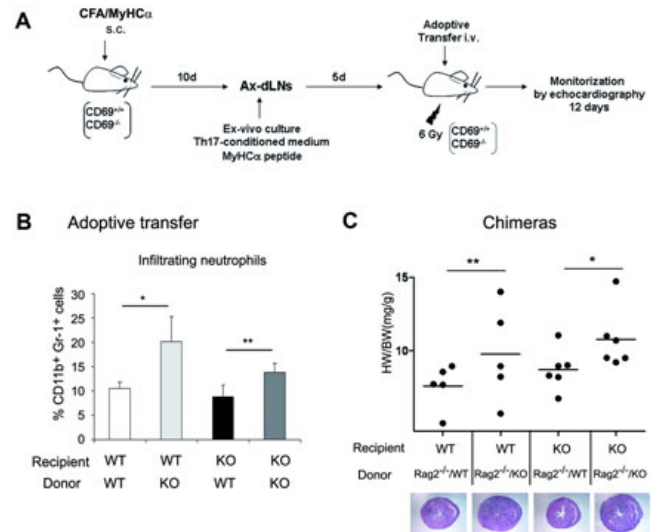


CD69 receptors are expressed on the membrane of T cells following activation (1). The cytoplasmic tail of CD69 associates with Jak3 and Stat 5 proteins, triggering phosphorylation of Stat5 and its translocation to the nucleus (2) where it can activate the transcription factor FoxP3, stimulating the differentiation of regulatory T cells (3). CD69 engagement can also induce expression of IL-2 and TGF- β . These cytokines may act in an autocrine manner to induce the differentiation of regulatory T cells (4). CD69 can inhibit the Th17 differentiation pathway through at least two mechanisms: CD69-activated Stat5 directly inhibits the translocation of Stat3 to the nucleus (5) and indirectly, via FoxP3 activation, antagonizes Stat3-mediated ROR γ t activation (6). APC, antigen presenting cell; TCR, T cell receptor; Treg, regulatory T cell; P, phosphorylation.

3 Vascular Biology and Inflammation



CD69 acts as a brake on the progression and severity of autoimmune myocarditis and the development of dilated cardiomyopathy (DCM). Our study paves the way to investigations into whether defects in CD69 expression or function influence the development of DCM in humans. These findings increase our knowledge of the development of myocarditis, providing a cellular and molecular basis for the development of novel therapies.



Adoptively transferred CD69^{-/-} Th17 cells can induce severe myocarditis in WT mice. (A) WT and CD69^{-/-} Th17 cells were produced by sensitizing mice to MyHC- α peptide followed by isolation from axillary-draining lymph nodes (Ax-dLNs) and in vitro derivation. The Th17 cells were then injected into either WT or CD69^{-/-} recipient mice. (B) Analysis of inflammation in recipient hearts. Bars represent the proportion of infiltrating neutrophils (CD11b⁺ and Gr-1⁺) in the myocardium 12 days after Th17 cell transfer. (C) CD69 WT and KO mice were lethally irradiated and reconstituted with a mix of bone marrow cells from RAG2^{-/-} plus CD69^{-/-} or RAG2^{-/-} plus CD69^{+/+} mice. Heart weight/body weight (HW/BW) ratios of individual chimeric mice after the induction of EAM are shown as dots; horizontal bars represent means. Representative myocardial cross sections are shown below the chart. Data correspond to the arithmetic mean and SD (n=6), and p values are indicated (one-way ANOVA and Bonferroni multiple comparisons test).



MAJOR GRANTS

- Ministerio de Ciencia e Innovación (SAF2008-02719)
- Ministerio de Ciencia e Innovación (RYC2006-2966)



SELECTED PUBLICATIONS

Cruz-Adalia A, Jiménez-Borreguero LJ, Ramírez-Huesca M, Chico-Calero I, Barreiro O, López-Conesa E, Fresno M, Sánchez-Madrid F, Martín P. **CD69 limits the severity of cardiomyopathy after autoimmune myocarditis.** *Circulation* (2010) 122: 1396-404

Martín P, Gómez M, Lamana A, Ramírez-Huesca M, Cruz-Adalia A, Ursa MA, Yáñez-Mo M, Sánchez-Madrid F. **CD69 association with Jak3/Stat5 proteins regulates Th17 cell differentiation.** *Mol Cell Biol* (2010) 30: 4877-89

Martín P*, Gómez M*, Lamana A*, Marín AM, Cortés JR, Ramírez-Huesca M, Barreiro O, Lopez-Romero P, Gutierrez-Vazquez C, de la Fuente H, Cruz-Adalia A, Sánchez-Madrid F. **The leukocyte activation antigen CD69 limits allergic asthma and skin contact hypersensitivity.** *J Allergy Clin Immunol* (2010) 126: 355-65

*Joint 1st authors

Barreiro O*, Martín P*, González-Amaro R and Sánchez-Madrid F. **Molecular cues guiding the inflammatory responses.** *Cardiovasc Res* (2010) 86: 174-82

*Joint 1st authors

Sandoval P, Loureiro J, González-Mateo G, Pérez-Lozano ML, Maldonado-Rodríguez A, Sánchez-Tomero JA, Mendoza L, Santamaría B, Ortiz A, Ruíz-Ortega M, Selgas R, Martín P, Sánchez-Madrid F, Aguilera A and López-Cabrera M. **PPAR- γ agonist Rosiglitazone protects peritoneal membrane from dialysis fluid-induced damage.** *Lab Invest* (2010) 90: 1517-32

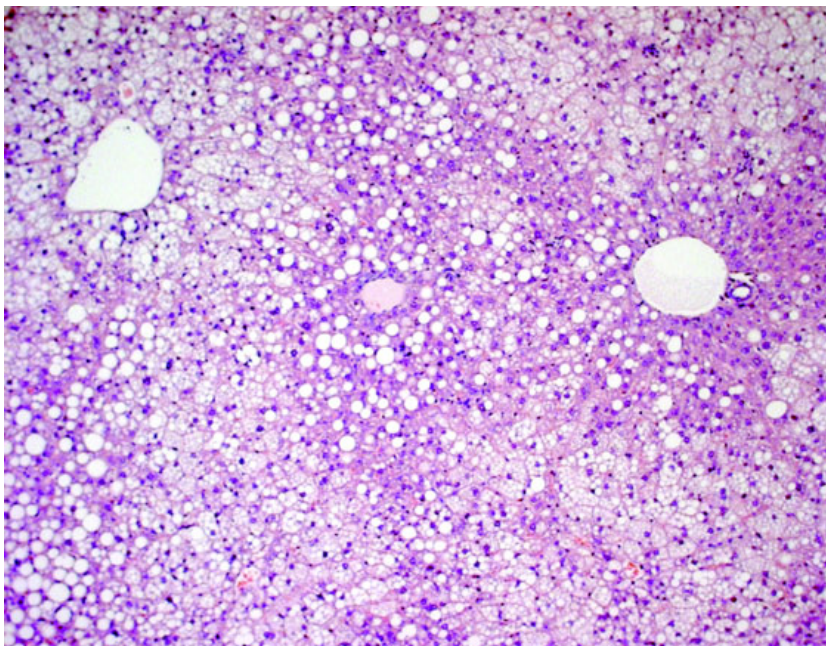
*Stress kinases in diabetes,
cancer and cardiovascular disease***Head of Laboratory:** *Guadalupe Sabio***Postdoctoral Researcher:** *Nuria Matesanz***Predocctoral Researchers:** *María Ángeles Verdugo
Elisa Manieri
Bárbara González
Edgar Bernardo***Technician:** *Luis Leiva***RESEARCH INTEREST**

Metabolic syndrome is a medical disorder defined by the co-occurrence of obesity, impaired glucose tolerance, dyslipidemia and hypertension. The condition is associated with proinflammatory and prothrombotic states, and the major clinical outcomes are cardiovascular disease and type 2 diabetes. Moreover, metabolic syndrome may be a predisposing factor for the development of some types of cancer, such as hepatocellular carcinoma.

The high cardiovascular risk associated with metabolic syndrome and type 2 diabetes suggests that common mechanisms are involved in the etiology of these conditions, and that disease parameters in both, might be improved by

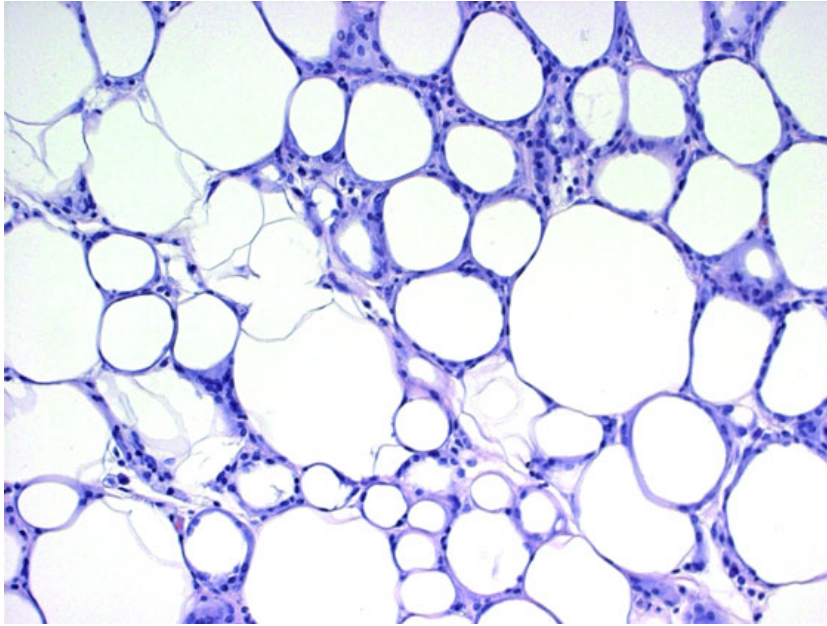
agents acting on the same therapeutic targets. Evidence from basic research suggests that one such target might be the stress activated protein kinases (SAPKs), an important family of kinases implicated in the transduction of stress signals into the cell.

Our recently formed group investigates the involvement of SAPKs in the development of cancer and atherosclerosis induced by obesity. Our research is conducted with a number of disease models in combination with whole genome and tissue-specific knockout mice, and has shown that the SAPK JNK regulates fat metabolism, obesity, dyslipidemia and glucose intolerance through its actions in various tissues.



Hematoxylin and eosin (H&E)-stained section of liver from C57Bl/6J mice fed a high-fat diet for 16 weeks.

3 Vascular Biology and Inflammation



H&E-stained section of epididymal fat from C57Bl6/J mice fed a high-fat diet for 16 weeks.



MAJOR GRANTS

- European Commission. European Research Council Starting Independent Researcher Grant (ERC-StG-260464)
- European Foundation for the Study of Diabetes (EFSD 0203)
- Papel de la obesidad en el desarrollo del cáncer hepático. Lóreal-Unesco



SELECTED PUBLICATIONS

Sabio G, Cerezo-Guisado MI, Del Reino P, Inesta-Vaquera FA, Rousseau S, Arthur JS, Campbell DG, Centeno F, Cuenda A. **p38gamma regulates interaction of nuclear PSF and RNA with the tumour-suppressor hDlg in response to osmotic shock.** *J Cell Sci* (2010) 123: 2596-604

Sabio G, Davis RJ. **cJun NH2-terminal kinase 1 (JNK1): roles in metabolic regulation of insulin resistance.** *Trends Biochem Sci* (2010) 35: 490-6

Sabio G, Cavanagh-Kyros J, Barrett T, Jung DY, Ko HJ, Ong H, Morel C, Mora A, Reilly J, Kim JK, Davis RJ. **Role of the hypothalamic-pituitary-thyroid axis in metabolic regulation by JNK1.** *Genes Dev* (2010) 24: 256-64

Sabio G, Kennedy NJ, Cavanagh-Kyros J, Jung DY, Ko HJ, Ong H, Barrett T, Kim JK, Davis RJ. **Role of muscle c-Jun NH2-terminal kinase 1 in obesity-induced insulin resistance.** *Mol Cell Biol* (2010) 30:106-15

Sabio G, Cavanagh-Kyros J, Ko HJ, Jung DY, Gray S, Jun JY, Barrett T, Mora A, Kim JK, Davis RJ. **Prevention of steatosis by hepatic JNK1.** *Cell Metab* (2009) 10: 491-8

CNIC-UAM COLLABORATIVE PROGRAM: *Intercellular communication in the inflammatory response*



Program Director: *Francisco Sánchez-Madrid*

Postdoctoral Researchers: *Olga Barreiro
Hortensia de la Fuente
Noa B. Martín Cofreces
Gloria Martínez del Hoyo
María Mittelbrunn
Vera Rocha*

Predocctoral Researchers: *Francesc Baixauli
Aránzazu Cruz
Cristina Gutiérrez
Giulia Morlino
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RESEARCH INTEREST

Intercellular communication is of critical importance for the innate and adaptive immune responses. Our group is interested in deciphering key communicative events during central processes of the immune response such as antigen presentation for T cell activation (immune synapse) and leukocyte trans-endothelial migration.

Cell-cell synapses are an exquisitely evolved mode of intercellular communication that is essential for neural and immune system functionality. The immune synapse (IS) is a transient, highly-specific and highly-ordered structure formed at the T cell–antigen-presenting cell (APC) interface through the reorganization of transmembrane and membrane-associated molecules. The tubulin cytoskeleton is rapidly directed toward the center of the IS through the translocation of the microtubule-organizing center (MTOC). This MTOC polarization brings the secretory apparatus into close apposition with the APC, thus providing the basis for polarized secretion. We are currently investigating the functional consequences of horizontal transfer of RNA-harboring exosomes from T cells to APCs at the IS, the role of the micro-RNA machinery in T cell activation, and the mechanisms of selective sorting of micro-RNAs and mRNAs

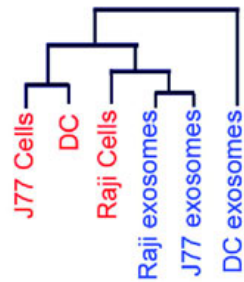
into exosomes during cognate interactions. We are also investigating horizontal transfer of genetic information during leukocyte-endothelium interactions.

MTOC translocation is also a mechanism for macromolecule transport and the nucleation of signaling and adapter molecules. We are interested in the regulation of MTOC-dependent mitochondrial polarization to provide a localized bioenergetic source for cytoskeletal rearrangements and exosomal delivery, in particular the role of the microtubule-polymerization promoter EB1. In addition, we are studying the role of the tubulin deacetylase HDAC6, a regulator of MTOC translocation, in inflammatory immune responses.

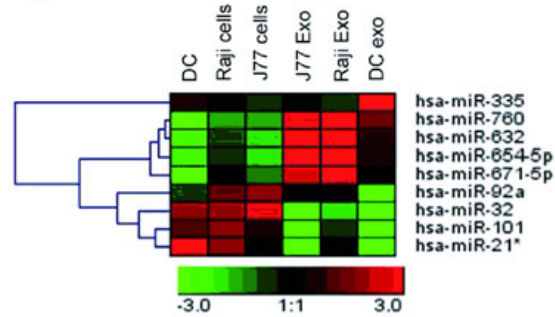
Another area of interest is the role of immunoregulatory molecules such as tetraspanins CD9 and CD81 and galectins 1, 3 and 9 in autoimmune disease. This is studied in models of two frequent autoimmune diseases: psoriasis, which is a Th1/Th17 inflammatory skin disease, and allergic asthma, which is mainly a Th2 chronic inflammatory disease. We also study the inflammatory response in a model of inflammation based on contact hypersensitivity, and have found in vivo imaging to be fundamental to our understanding in this area.

3 Vascular Biology and Inflammation

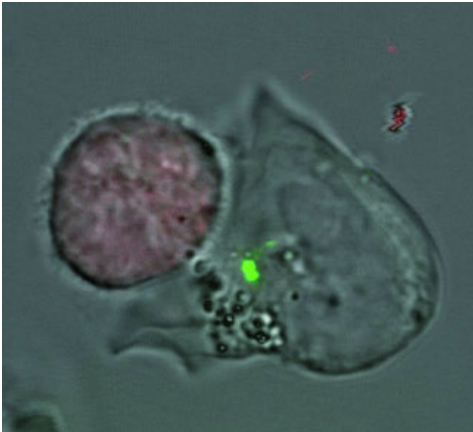
a Hierarchical clustering



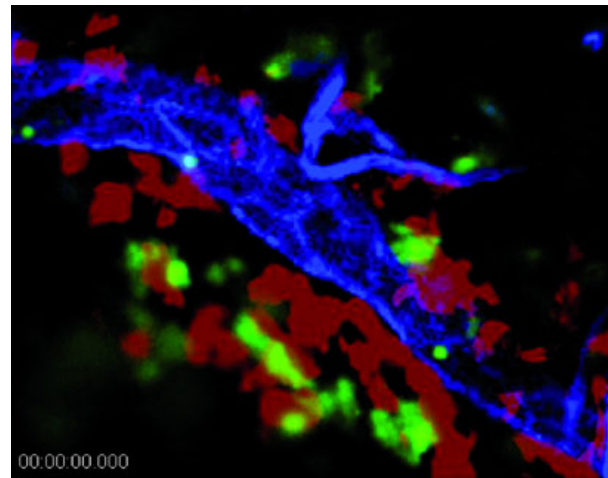
b



a, Microarray analysis of exosomal miRNAs and the miRNAs of their respective donor cells. The panel shows the hierarchical clustering of the vsn-normalized array data in the log₂ scale averaged per biological replicate for each origin (exosomes/cells) and cell type (DC: dendritic cells; J77: Jurkat-derived J77 T cell line and Raji: Raji B cell line). **b**, Heatmap of the vsn-normalized data for selected miRNAs.



Disruption of AKAP450 function impairs MTOC translocation towards the immune synapse. Cell conjugates were formed between J77 cells overexpressing C-terminally GFP-tagged AKAP450 (green) and SEE-pulsed Raji APCs (red). MTOC position (GFP signal) was monitored by confocal fluorescence microscopy.



Intravital microscopy image showing leukocyte-endothelium interactions in an inflamed area (mouse dermis). To allow visualization, we adoptively transferred GFP+ hematopoietic cells to a C57Bl6 recipient mouse, in which the vasculature was traced in blue using an anti-CD31 antibody and perivascular cells were stained red.



MAJOR GRANTS

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SELECTED PUBLICATIONS

Robles-Valero J, Martín-Cófreces NB, Lamana A, Macdonald S, Volkov Y, [Sánchez-Madrid F](#). Integrin and CD3/TCR activation are regulated by the scaffold protein AKAP450. *Blood* (2010) 115: 4174-84

[Barreiro O](#), Martín P, González-Amaro R, [Sánchez-Madrid F](#). Molecular cues guiding inflammatory responses. *Cardiovasc Res* (2010) 86: 174-82

Martín P, Gómez M, Lamana A, [Cruz-Adalia A](#), [Ramírez-Huesca M](#), Ursa MA, Yáñez-Mo M, [Sánchez-Madrid F](#). CD69 association with Jak3/Stat5 proteins regulates Th17 cell differentiation. *Mol Cell Biol* (2010) 30: 4877-89

Martín P, Gómez M, Lamana A, Marín AM, Cortés JR, [Ramírez-Huesca M](#), [Barreiro O](#), López-Romero P, [Gutiérrez-Vázquez C](#), de la Fuente H, [Cruz-Adalia A](#), [Sánchez-Madrid F](#). The leukocyte activation antigen CD69 limits allergic asthma and skin contact hypersensitivity. *J Allergy Clin Immunol* (2010) 126: 355-65

[Baixauli E](#), [Martín-Cófreces NB](#), [Morlino G](#), Carrasco YR, Calabia-Linares C, Veiga E, Serrador JM, [Sánchez-Madrid F](#). The mitochondrial fission factor dynamin-related protein 1 modulates T-cell receptor signalling at the immune synapse. *EMBO J* (accepted)

Immunobiology of inflammation

Head of Laboratory: *David Sancho Madrid*

Predocctoral Researcher: *Noelia Blanco Menéndez*

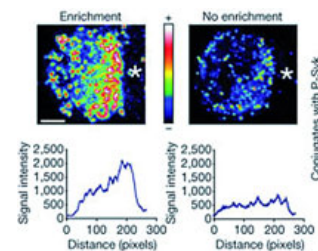
Masters Student: *María Martínez López*

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**RESEARCH INTEREST**

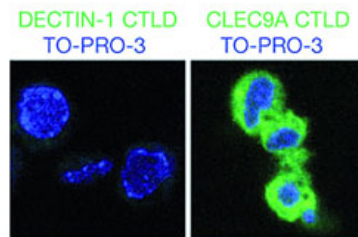
Impaired clearance of apoptotic cells results in the accumulation of secondary necrotic corpses, with profound immune consequences. Cell death triggers the macrophage inflammatory response, which normally contributes to tissue repair but under certain conditions can induce a state of chronic inflammation that is the basis of many diseases. Necrosis sensing by dendritic cells (DCs) might explain adaptive immunity in seemingly infection-free situations such as autoimmunity. Myeloid C-type lectin receptors (CLRs), such as Mincle in macrophages and CLEC9A (DNDR-1) in DCs, have been identified as receptors for necrotic cells that couple to the tyrosine kinase Syk, which in turn can trigger innate and adaptive immune responses.

Our hypothesis is that recognition of cell death by Syk-coupled CLRs in myeloid cells might lie at the root of immune pathologies associated with an accumulation of dead cells. We are characterizing signaling and gene induction via CLEC9A as a model of innate sensing of necrotic cells by DCs. We are also investigating the role of Syk signaling and Syk-coupled receptors in myeloid cells, in models of autoimmunity and of immune responses to dead tumor cells after chemotherapy. CLEC9A and Mincle are prime candidate mediators of the response to dead cells in DCs and macrophages, but our preliminary findings indicate that Syk deficiency has a more profound effect than CLEC9A deficiency on the sensing of necrosis by DCs, suggesting that additional receptors are involved. The third strand of our research is thus focused on the identification of new Syk-coupled receptors that recognize necrosis in myeloid cells.



CLEC9A-dependent enrichment for phospho-Syk at the contact area between DCs and dead cells. DC-dead-cell conjugates were formed and stained for P-Syk. P-Syk concentrates in the contact area of conjugated cells only in the presence of CLEC9A.

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CLEC9A ligand is preformed and intracellular. Fixed and permeabilized mouse embryonic fibroblasts were labeled with the ligand binding domain monomers DECTIN-1 (negative control) or CLEC9A, in green, and counterstained with TO-PRO-3 (nuclear dye) before confocal microscopy. Original magnification, x630.



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Poulin LF, Salio M, Griessinger E, Anjos-Afonso F, Craciun L, Chen JL, Keller AM, Joffre O, Zelenay S, Nye E, Le Moine A, Faure F, Donckier V, [Sancho D](#), Cerundolo V, Bonnet D, Reis e Sousa C. **Characterization of human DNGR-1+ BDCA3+ leukocytes as putative equivalents of mouse CD8alpha+ dendritic cells.** *J Exp Med* (2010) 207: 1261-71

Joffre OP, [Sancho D](#), Zelenay S, Keller AM, Reis e Sousa C. **Efficient and versatile manipulation of the peripheral CD4+ T-cell compartment by antigen targeting to DNGR-1/CLEC9A.** *Eur J Immunol* (2010) 40: 1255-65

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