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BOOK OF ABSTRACTS

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cytoplasmic content to the lysosome for degradation, has been linked to the development of numerous inflammatory conditions (*3*). While there is ample evidence of immune cell autophagy-related genes regulating inflammation, less is known about the role of EC autophagy in this context. Here, we explored the role of microvascular EC autophagy in neutrophil trafficking within multiple acute models of inflammation.

Canonical autophagy involves the formation of dedicated double–membrane vesicles commonly known as autophagosomes. These organelles can be identified by their association with the membrane-bound lipid modified form of microtubule-associated protein light chain 3 (LC3) through development of characteristic LC3-punctae. Using high-resolution confocal microscopy, we found that inflamed postcapillary venular ECs exhibited enhanced levels of LC3-puncta that localised exclusively to EC borders, an event aligned temporally with the peak of neutrophil trafficking. Furthermore, confocal intravital microscopy revealed significantly exaggerated and faster neutrophil transendothelial migration across autophagy deficient ECs, while pharmacological induction of autophagy inhibited neutrophil migration. Mechanistically, autophagy machinery regulated the remodeling of EC junctions and expression of key EC adhesion molecules, facilitating their intracellular trafficking and degradation. Since lack of EC autophagy led to excessive neutrophil infiltration in multiple inflammatory models, our results identify EC autophagy as an essential cellular process to limit physiological inflammation.

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0391-P

A carbohydrate-binding trimeric fragment of lung surfactant protein SP-A neutralizes cytotoxic and pro-inflammatory effects of cathelicidin on alveolar epithelial cells

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Human cathelicidin (LL-37) is a host defense peptide with direct antimicrobial activity against several pathogens. However, LL-37 also can trigger tissue injury through binding to host membranes, causing cytotoxic or proinflammatory effects. LL-37 is secreted by epithelial and immune cells of the skin, intestine, ocular system, and lung. LL-37 levels rise in airways of chronic obstructive pulmonary disease patients, contributing to chronic inflammation. Surfactant protein SP-A is secreted by the alveolar epithelium and has essential immune functions in the lung. It is a large oligomeric protein assembled in multiples of three subunits, which contain a collagen-like domain and globular recognition domains. The objective of this study was to investigate whether either human SP-A or a trimeric recombinant fragment of the protein, which lacks most of the collagen domain (rfhSP-A), is involved in local regulation of LL-37 activity. To address this question, we studied the interaction of LL-37 with SP-A and rfhSP-A by tryptophan fluorescence and the effects of these proteins on LL-37 antimicrobial and cytotoxic activities. We found that both SP-A and rfhSP-A bound to LL-37 with high affinity in physiological conditions (K_d = 0.45 ± 0.01 nM for SP-A and K_d = 1.22 ± 0.73 nM for rfhSP-A). Such interactions result in reduction of LL-37-induced cytotoxicity and inflammation in alveolar epithelial cells. However, LL-37 antimicrobial activity against respiratory pathogens (*Klebsiella pneumoniae* K2, *Pseudomonas aeruginosa* O1, and nontypeable *Haemophilus influenzae)* was not affected by either SP-A or rfhSP-A. These results demonstrate that SP-A plays a protective role in reducing LL-37's cytotoxic and inflammatory actions, which depends on SP-A's globular/neck domains. Our studies also suggest a potential therapeutic effect of rfhSP-A on chronic inflammatory lung diseases characterized by elevated LL-37.

0393-P

Inflammasome inhibition and breast cancer

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Inflammasomes are intracellular multiprotein platforms of the innate immune system that are activated in response to pathogens or intracellular damage. The formation of ASC specks, initiated by different inflammasome receptors, promotes the recruitment and activation of procaspase-1, thereby triggering pyroptotic inflammatory cell death and pro-inflammatory cytokine release.

Here, we describe a *pan* inflammasome inhibitor, MM01 that interferes with ASC speck formation and inhibits inflammation *in vivo*. This inhibitor could be useful for the potential treatment of multifactorial diseases involving the dysregulation of multiple inflammasomes and also as a tool to explore the role of inflammation in the progression of cancer.

Inflammation is a well-established hallmark of cancer. Tumor development and progression not only depends on genetic alterations of tumor cells, but also on the inflammatory tumor microenvironment. In breast cancer, two controversial situations have been observed: the elimination of the inflammasome components and the associated reduction in pro-inflammatory cytokine release decreases tumor size and metastasis and on the other hand, the lack of inflammatory response triggers a more aggressive phenotype of the tumor. Features that tip the balance towards one type or another of response are unknown. Our aim is to identify potential biomarkers capable to classify tumors based on their response to anti-inflammatory therapies using an in vitro assay and our pan inflammasome inhibitor as predictive tool.

0396-R/M-P

BH3 mimetics sensitize bladder cancer cells to cisplatin treatment

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Bladder cancer is the ninth most common cancer in the world. About 30 percent of cases appear as muscle invasive carcinomas, which carry an increased risk of metastatic disease. These carcinomas may require radical surgery and chemotherapy with gemcitabine and cisplatin, and relapsed or refractory cases may need second-line therapies. Platinum-based drugs are currently used for the treatment of various solid cancers, however, their use is mainly limited by chemoresistance and adverse effects in normal tissues[1-3].

Bcl-2 family proteins are a group of structurally related proteins composed of pro-apoptotic members such as Bax and Bak, and pro-survival members such as Bcl-xL, Bcl-2 or Mcl-1. Overexpression of pro-survival members can lead to resistance to chemotherapeutic drugs, so the development of drugs targeting these molecules is becoming increasingly a strategy to overcome resistance to first-line cancer therapy. Two of these inhibitors, commonly known as BH3 mimetics, are Obatoclax (targeting all Bcl-2 family pro-survival proteins) and ABT-737 (selectively targeting Bcl-2, Bcl-xL and Bcl-w). The aim of this study was to analyse different combinations of these BH3 mimetics with cisplatin to identify synergies between these treatments and to develop new therapeutic strategies[4-7].

We show that the combination of ABT-737 or Obatoclax with cisplatin is able to reverse cisplatin resistance in muscle-invasive bladder cancer cells. In HT1197 cells we observe sensitisation to cisplatin after combination with ABT-737, these cells show cisplatin resistance mediated, at least in part, by autophagy. In HT1376 cells we observe sensitisation to cisplatin after combination with Obatoclax. We have also observed that the combination of Obatoclax and ABT-737 induces cell death in these cell lines. Combinations of BH3 mimetics and cisplatin may be alternative therapies in muscle-invasive bladder cancer and Bcl-2 family proteins may be predictive markers of response.

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0410-R-P

Anti-inflammatory potential of aeroplysinin-1, a bioactive compound isolated from the sponge Aplysina aerophoba

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Aeroplysinin-1 (Apl-1) is a brominated compound isolated from the marine sponge Aplysina aerophoba that has been shown to possess bioactive effects with a broad spectrum of action in *in vitro* and *in vivo* assays. Included in its pleiotropic activity are anti-tumor, anti-angiogenic, pro-apoptotic [1] and anti-oxidant effects [2], making Apl-1 a natural compound with very promising properties for its use as a potential therapeutic agent. In addition to the aforementioned effects, our group explored the role of Apl-1 in inflammation, a process related to numerous highly prevalent pathologies, such as cancer and atherosclerosis, showing the first evidence of its anti-inflammatory potential [3].

In this work, our group delves into the anti-inflammatory effect of Apl-1 in the context of vascular endothelium and provides new data regarding the molecular mechanism underlying this activity. The characterization of the mechanism of action points to the modulatory effect of Apl-1 on pathways involved in endothelial activation during the development of inflammation, experimental evidence that opens the door to the potential use of this compound as an anti-inflammatory agent.

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