PROPUESTA CIENTÍFICA - *SCIENTIFIC PROPOSAL*

Neonatal Gut Microenvironment: The role of Vacuolated Enterocytes on Intestinal Communication, Homeostasis and Disease

(NEOGUT)

1 PROPOSAL OUTLINE

The intestine is one of the central organs of the body: it controls the host's metabolism and physiology and presents a critical function of immunity through the constant interaction with commensal microbes and pathogens. This interaction is essential for obtaining its functional balance, known as **intestinal homeostasis**. Intestinal homeostasis is consolidated throughout the individual's life but must be established at birth when exposed to microbes[1]. The immune system must balance protecting the newborn from pathogens while ensuring tolerance to beneficial microbes that are critical for health. Alterations of gut homeostasis can lead to the development of chronic inflammatory diseases such as inflammatory bowel diseases (IBD) and increase the risk of colorectal cancer (CRC). Multiple risk factors influence the development of CRC; however, the influence of the neonatal microenvironment to IBD and CRC is unknown.

Interestingly, the establishment of this initial neonatal communication is based on the presence of neonatal vacuolated enterocytes (NEs) with high absorptive capacity, which are replaced by adult enterocytes (IEC) at weaning. NEs present a large lysosome in their cytoplasm necessary to internalize mother-derived macromolecules, digest the nutrients from breast milk and process other critical metabolites[1–4]. Our recent results have demonstrated NEs have a very high metabolic activity that largely depends on their enormous autophagic activity, and the presence of a conspicuous mitochondrial network, both associated with the giant lysosome (Herranz et al, submitted). Indeed, the intestinal-specific neonatal alteration of autophagy and mitochondria activity markedly affects the gut microenvironment, producing a generalized disorganization of the lysosomal activity of NEs, a significant alteration of epithelial patterning, an increase in inflammation markers and anticipated weaning transition. We hypothesize that the function of the NEs must be essential for establishing intestinal homeostasis, not only at the neonatal stages, but also in adults. This function must be modulated by the mitochondrial-lysosomal activity of NEs, establishing a metabolic communication regulating the microbiota and mucosal immune system. The goal of NEOGUT proposal is to characterize the importance of neonatal NEs for establishing intestinal homeostasis and their importance in developing human diseases such as IBD and CRC.

2 Scientific, economic and social impact.

NEOGUT focuses on understanding the function and importance of neonatal vacuolated enterocytes (NEs) in establishing intestinal homeostasis and their role in the development of IBD (ulcerative colitis and Crohn's disease) and CRC. The hypothesis is that mitochondrial-lysosomal activity within NEs is critical for regulating the microbiota and the mucosal immune system. The project will explore how the gut manages symbiotic relationships with microbes, the impact of nutrition deficits in early life, and the specialized role of NEs in protein absorption and processing. It will investigate the highly metabolic activity of NEs, their autophagic activity, and mitochondrial networks in relation to gut microenvironment and weaning transitions.

Finally, the study will investigate the influence of neonatal enterocyte metabolism on the development of the gut microbiome and immune system maturation, which is crucial for establishing a lifelong microbiome and immune profile. It seeks to understand the consequences of metabolic disruptions on the progression of IBD, aiming to provide insights into early-life interventions to prevent disease onset.

Overall, the project aligns with national and European research strategies, **emphasizing excellence and interdisciplinary research**, and could lead to significant advances in understanding vertebrate development, adult gut homeostasis, and the discovery of

pharmaceutical targets in diseases like IBD and CRC. This research also highlights the importance of neonatal health, leading to increased public awareness and advocacy for early-life health interventions.

3. Training plan (an specific Development Training Plan for PhD students).

I have supervised both undergraduate and Ph.D. students since I was a Ph.D. student myself. During my post-doc stages (at UCSF), I continued supervising students. Since my group has formed at the CBMSO, it has received particular attention from under-graduate students of several universities in Spain. However, few of them (due to a space limitation in our lab) have been accepted as part of the university training. Currently, there are 5 Ph.D. students (G. Herranz, G. Baonza, Diego Alonso, L. Akintche and A. Sanchez de la Cruz) and one master students (C.). During the last five years, 4 Ph.D. students have successfully graduated with the maximum qualification and left our group (M. Hachimi, M. Bosch-Fortea, M. D. Barea and S. Gómez). They have joined excellent international groups as post-doc (see the section below). One student (Gonzalo Herranz) is the last part of his Ph.D. program and will leave the group during 2024. To maintain a perfect balance between experienced scientist (postdocs) and new future talents, I recruited two more Ph.D. students (L. Akintche and A Sanchez) through a Marie-Curie Doctoral Network (Surfex). I am also requesting one FPI fellowship, since the possibility of incorporating a new Ph.D. student will give continuity to our research line and is also of paramount importance for the proper development of this grant proposal.

During his/her Ph.D., the new students will learn both basic molecular biology techniques, organoids and scRNA-seq analysis, protein-protein interactions, among other techniques. An essential aspect of the students' scientific training in my lab is that their work includes animal models using developmental biology, cell and molecular biology, and biomechanics. Experience in multiple animals will help students develop a broad view of developmental, evolutionary, and cell biology by comparing mechanisms that function during different species' development. I supervise new Ph.D. student activity with regular meetings. I intend for the new Ph.D. students to participate in – at least – one national/international event per year, which is an essential additional training/educational aspect. He/she will benefit from participating in such events, which will allow us to present recent results in front of experts in our research field and establish connections with scientists of different labs and from different countries. Notably, most of the students in my lab have been either the first author or co-author of several publications, which allow them to apply for post-doc fellowship and join excellent international labs. The training provided within my group is not only limited to Ph.D. students. Indeed, former post-docs in the lab have got important academic and industrial positions in Europe.

The CBMSO has a Ph.D. Training Plan based on its participation either as an organizer or as a participant in several masters and doctoral programs of the Universidad Autónoma de Madrid (UAM) within which campus our Center is located. Since its foundation, the CBMSO is known for its research in the area of Molecular Biology and currently belongs to *The campus of International Excellence* (CEI) CSIC-UAM constitutes the most critical research nucleus in Spain in the area of Molecular Biosciences, according to the impact of scientific publications. CBMSO has participated as organizers in the Postgraduate Programme of Molecular Biosciences of the UAM, which includes the Doctorate in Molecular Biosciences and the Masters in Biotechnology, Biomolecular and Cellular Dynamics and Molecular Biomedicine. All CBMSO doctorates have training plans and extensive monitoring approved by competent agencies of verification. They are subjected to a periodic accreditation procedure that ensures the formative activities' quality in terms of research training and the program's management.

Since 2007 I am involved in teaching at the UAM Master School. Moreover, several Master students have been accepted for a stage in our lab as a training experience. As an external professor, I have further participated in several other Ph.D. Curses are organized by other Universities and institutes (CNIC, CNIO, Institut Pasteur, Institut Curie, San Raffaelle Ph.D. School of Milan). I am co-supervising several Ph.D. students in European research centers. On the other hand, the CBMSO is also involved in other Doctorates such as the masters and doctoral degrees in Biology, and Physics of Condensed Matter, Nanoscience, and Biophysics.