

Cell diversity of invertebrate hemocytes and its relevance for development, immune and regenerative functions

Background The immune system is widely recognized as the repository of the body's capacity to counteract the presence of pathogens as well as non-pathogenic non-self-components. It relies principally on circulating blood cells and, crucially, it enables animals to recognize and either tolerate or get rid of non-self-entities, allowing them to adapt to the environmental antigenic ecospace (Capri *et al.*, 2014). Much of what we know about immunology and hematopoiesis comes from vertebrate models, which present distinct lymphoid and myeloid circulating cell lineages. Yet, several aspects of human immunity, especially those concerning the innate component, remain obscure. Evidence in the last decades have been indicating that cells such as macrophages, neutrophils and mast cells can manifest substantial plasticity (Galli *et al.*, 2011). Similarly, recent functional and molecular studies on invertebrate hemocytes, which are categorized based on their morphology or phagocytic activity, have highlighted that the same morphotypes have different gene expressions and cell functions (Sacchi *et al.*, 2024). This added an unexpected layer of complexity to the invertebrate immune systems. Moreover, growing evidence has challenged the traditional view of immune cells as agents devoted solely to defence against pathogens (Arinda *et al.*, 2022). During embryonic and post-embryonic development, these cells are increasingly recognized as active participants in tissue morphogenesis, shaping and maintaining structural and functional integrity of developing tissues (Morimoto *et al.*, 2019; Krejčova *et al.*, 2024). Among invertebrates, information on hemocyte lineages is available for insects and crustaceans, while in molluscs little is known about the tissue context and molecular mechanisms in which these cells can proliferate and mature, and to date no specific cell markers to track hemocytes during their development and differentiation are available (Pila *et al.*, 2016). The laboratory-bred snail *Pomacea canaliculata* (*Pc*) can address the drawbacks of molluscan models. *Pc* can undergo repeated hemolymph withdrawals (Accorsi *et al.*, 2014) and recent unpublished data showed it is able to dynamically control hemocyte number and morphotype proportions. *Pc* exhibits regeneration capabilities (Bergamini *et al.*, 2023, Accorsi *et al.*, 2025), direct development and a long lifespan, all valuable traits for studying hemocyte diversity.

Aims and activities Leveraging the increasing availability of omic data for *Pc*, during the first year of my PhD, I will start from the available databases to identify hemocyte-enriched markers and to characterize *Pc*-homologous of well-known hematopoietic factors. I will then analyze the expression of hemocyte markers and hematopoietic factors across embryonic and adult transcriptomic datasets to obtain preliminary *in silico* expression profiles. I will then validate top candidate genes via RT-qPCR on adult tissues and in pre- and post-hatching juveniles. During the second year, I aim to document the embryonic origin of hemocytes via *in-situ* techniques, using probes targeting the markers identified during the first year. Direct development of *Pc* allows developmental processes to be studied in absence of larval metamorphosis. In addition, the protocol to cultivate embryos *ex-ovo* is also available (Accorsi *et al.*, 2025). This developmental study, beside filling a significant gap in the knowledge of molluscan developmental immunology by defining the timing of hemocyte specification, offers a valid biological context for investigating hemocytic involvement in tissue morphogenesis and homeostasis. I will pursue this goal through functional approaches. Finally, I will use flow cytometry to monitor hemocyte morphotypes during growth, and I will work to develop protocols that allow for the sorting of subpopulations carrying specific hemocyte markers.

In conclusion, my project focuses on a fundamental and challenging biological question of developmental and comparative immunology. It aims to understand how the immune cells differentiate and act during the snail development, switching between developmental and defensive functions. This knowledge would offer a powerful framework for redefining immunity as an integrated system that supports growth and long-term tissue maintenance, rather than merely acting as a protective barrier. Additionally, as *P. canaliculata* is listed among the world's 100 most invasive species and is an intermediate host for a human parasite, improved knowledge of its hemocytes during development and growth may provide valuable insights for sustainable control strategies and the development of an ethically approved animal model for biomedical studies.

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