Polarization of vertebrate immune cells is commonly observed in reactions related to inflammation or tissue repair. The polarization process has been well known in the case of vertebrate macrophages (M), which, under the influence of lipopolysaccharide (LPS), gamma interferon (IFN-γ) or tumor necrosis factor alpha (TNF-α), exhibit classic M1 polarization and a strong pro-inflammatory effect associated with the elimination of pathogens. In contrast, anti-inflammatory cytokines (IL-4, IL-13, IL-10) and cortisol polarize macrophages into an alternative, anti-inflammatory M2 phenotype, regulating the inflammatory response to avoid host tissue damage, which is associated with promoting tissue repair (healing and regeneration). A typical difference between M1 and M2 macrophages is their L-arginine metabolism, involving either inducible nitric oxide synthase (iNOS), which catalyzes the formation of nitric oxide (NO) in classically activated macrophages, or increased expression of arginase 1 altering L-arginine metabolism towards production L-ornithine and polyamines, which consequently blocks iNOS in alternatively activated macrophages. However, L-ornithine is needed, among others, for cell proliferation and collagen formation. The fact that arginase is involved in the polarization process in mammals and lower vertebrates is well known and described, but its role in phagocyte polarization in invertebrates is not explored.

Invertebrate organisms that have very efficiently functioning cellular and humoral immune mechanisms that allow them to survive in a natural environment rich in pathogens include, among others: earthworms. Their immunocompetent cells - coelomocytes - are divided into two basic populations, amoebocytes and eleocytes. Amebocytes are phagocytic cells and, like mammalian macrophages, migrate to the site of infection, absorb or surround pathogens using the activity of the prophenoloxidase (proPO) cascade, and produce reactive oxygen species (ROS) and nitric oxide (NO). Eleocytes are mainly involved in the synthesis of cytotoxic and antibacterial molecules. Interestingly, earthworms, having only an innate immune system, have a well-developed ability to regenerate lost body segments. The regenerative processes in these organisms have been well studied from a morphological perspective, but the molecular and immunological basis is largely still under investigation. It is known that rapidly increasing environmental pollution, including soil pollution, significantly affects the viability, reproduction, and also the resistance of earthworms. The pollutants whose concentration in the soil is currently increasing significantly include nanoparticles, including silver nanoparticles (AgNPs), which are currently among the most commonly used nanomaterials in medicine, industry, households and commercial applications. Importantly, dressings coated with silver nanoparticles are widely used in the treatment of acute and chronic wounds, and the potential of the ability of nanoparticles to change the polarization of responses is of interest to many researchers. However, whether exposure to AgNPs can influence the course of the inflammatory reaction and wound healing in invertebrates remains ambiguous. The aim of the project will be to demonstrate whether and by what factors earthworm coelomocytes (C) can become polarized and have a pro- (C1) and anti-inflammatory (C2) character, as well as to link polarization with the healing and regeneration processes after amputation of the rear body segments. At the same time, we plan to demonstrate how nanosilver compounds can influence the immune responses of earthworms, including the healing and regeneration process. To answer our questions, we will conduct in vitro and in vivo experiments on earthworms of the species *Eisenia andrei*. In part of the in vitro studies, we will verify the direct effect of various immunostimulants on coelomocytes, imitating viral, bacterial and parasitic infections and/or the polarization of coelomocytes with AgNPs. In turn, in vivo research will focus on the process of wound healing/regeneration in animals that have had the last sections of their bodies amputated.

We believe that this research will expand knowledge about earthworm immunity and the evolutionary behaviour of immune mechanisms, including the polarization of immunocompetent cells and their role in regenerative processes. Our research will also allow for a more detailed look at the impact of silver nanoparticles on earthworms and their immune system. Additionally, as nanoparticles become common across industries, understanding their impact on fundamental biological processes is essential to assessing the potential risks of their use.