Funded Positions for PhD Students in Comparative Immunology

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The Grayfer Lab

Amphibian innate immunity: Molecular regulation of *Xenopus laevis* macrophage development and antimicrobial defenses.

Background information: Amphibians possess considerably less efficient adaptive immune responses, as compared to mammals. Thus, these animals must rely more heavily on their innate immune defenses. It is noteworthy that cells belonging to the macrophage lineage are integral to all vertebrate innate immune and antimicrobial responses, and yet are often also infiltrated by invading infectious agents, resulting in pathogen persistence and dissemination. This kind of host immune cell-pathogen relationship is exemplified by the infection of amphibians with the Frog Virus 3 ranavirus, wherein distinct lineages of amphibian (*Xenopus laevis*) macrophages confer susceptibility and resistance to this infectious agent. In fact, the success of any given antimicrobial response often hinges on pertinent macrophage development and functional polarization. Accordingly, the Grayfer lab is interested in elucidating the specific immunological strategies by which cold-blooded vertebrate species such as amphibians coordinate their macrophage ontogeny and antimicrobial defenses.

Rationale and objectives: Our work has demonstrated that different *X. laevis* growth and activation factors elicit macrophages with strikingly distinct antimicrobial capacities. Investigation into how these cells develop and are immunologically regulated will provide new insights into both the sources of vertebrate macrophage functional heterogeneity, as well as the facets of amphibian susceptibility and resistance to emerging pathogens such as Frog Virus 3.

Presently, the scope of our research is focused on delineating the molecular mechanisms governing *X. laevis* macrophage development; the identification and characterization of growth and activation factors that contribute to these processes and to more precisely defining the roles of different frog macrophage lineages during antimicrobial responses against infectious agents such as Frog Virus 3.

References:

Grayfer L, Robert J. (2015) Distinct functional roles of amphibian (*Xenopus laevis*) colony-stimulating factor-1- and interleukin-34-derived macrophages. J Leuk Biol. doi: jlb.4AB0315-117RR

Grayfer L, De-Jesús Andino F, Robert J. (2015) Prominent amphibian (*Xenopus laevis*) tadpole type III interferon response to the Frog Virus 3 ranavirus. J Virol 89: 5072-5082.

Grayfer L, Robert J. (2014) Divergent antiviral roles of amphibian (*Xenopus laevis*) M-CSF- and IL-34-derived macrophages. J Leuk Biol 96: 1143-1153.

Grayfer L, Edholm ES, De-Jesús Andino F, Chinchar VG, Robert J. (2014) Ranavirus host immunity and immune evasion. In: Ranaviruses: lethal pathogens of ectothermic vertebrates. Gray MJ (ed) Springer.

Grayfer L, De-Jesús Andino F, Robert J. (2014) The amphibian (*Xenopus laevis*) type I interferon response to Frog Virus 3: new insight into ranavirus pathogenicity. J Virol 88: 5766-5777

Grayfer L, Robert J. (2013) Colony-stimulating factor-1-responsive macrophage precursors reside in the amphibian (*Xenopus laevis*) bone marrow rather than the hematopoietic sub-capsular liver. J Innate Immun 5: 531-542

The Smith Lab

Invertebrate Immunology: Innate immune responses to pathogens and immune diversification in the purple sea urchin.

Background: Marine invertebrates share their environment with large numbers of bacteria and viruses that are present in sea water and the marine substrate. The genome of the purple sea urchin, *Strongylocentrotus purpuratus*, has shown that this invertebrate has a highly sophisticated innate immune system with multi-gene families of innate immune genes. We are evaluating one of these immune gene families called the *Sp185/333* genes that have an estimate of up to 60 genes per family, per genome. The genes are small, with only two exons, have multiple repeats and blocks of sequence called elements. The genes are clustered in the genome, and each is surrounded by microsatellites. Significant sequence diversification among the genes may be a combination of gene duplication and/or deletion, segmental duplication, as well as gene conversion. The encoded proteins, called SpShapeshifters, are further diversified through RNA editing and putative post-translational modifications. Sea urchins have up to 260 SpShapeshifter proteins that are present in suites of isoforms that are different among individual animals. The SpShapeshifter proteins are secreted, bind to marine microbes and yeast, and augment phagocytosis by the sea urchin phagocytes. A recombinant SpShapeshifter protein binds to a subset of pathogen associated molecular patterns with high affinity and specificity. The diversity and expression patterns of the *Sp185/333* gene system and encoded proteins are likely to be a major pathogen effector system in the sea urchin.

Rationale and objectives: Because echinoderms are phylogenetically related to chordates, investigations of the sea urchin immune system may provide insights into the evolutionary history of the immune system in higher deuterostomes. The goals of our research are to understand the diverse *Sp185/333* system, including the breadth of protein function, the source(s) of gene sequence diversity, the locations and activities of promoters that regulate expression for the different *Sp185/333* genes, and the mechanisms of SpShapeshifter protein binding.

References: (all are available from http://biology.columbian.gwu.edu/l-courtney-smith)

Majeske, AJ, M Oren, S Sacchi, **LC Smith**. 2014. Single sea urchin phagocytes express messages of a single sequence from the diverse *Sp185/333* gene family in response to bacterial challenge. *Journal of Immunology,* 193:5678-5688.

Majeske, AJ, T Oleksyk, **LC Smith**. 2013. The *Sp185/333* immune response genes and proteins are expressed in cells dispersed within all major organs of the adult purple sea urchin. *Innate Immunity*, 19(6):569-587.

Smith, LC 2012. Innate immune complexity in the purple sea urchin: diversity of the *Sp185/333* system. *Frontiers in Immunology* 3:70.

Miller, CA, KM Buckley, RL Easley & **LC Smith**. 2010. An *Sp185/333* gene cluster from the purple sea urchin and putative microsatellite-mediated gene diversification. *BMC Genomics,* 11:575.

Buckley, KM, S Munshaw, TB Kepler & **LC Smith**. 2008. The *185/333* gene family is a rapidly diversifying host-defense gene cluster in the purple sea urchin, *Strongylocentrotus purpuratus*. *Journal of Molecular Biology*, 379:912-928.

Buckley, KM, DP Terwilliger, **LC Smith**. 2008. Sequence variations in *185/333* messages from the purple sea urchin suggest post-transcriptional modifications to increase immune diversity. *Journal of Immunology*, 181:8585-8594.

Brockton V., JH Henson, DA Raftos, AJ Majeske, Y-O. Kim & **LC Smith**. 2008. Localization and diversity of 185/333 proteins from the purple sea urchin, *Strongylocentrotus purpuratus* (Stimpson); unexpected protein size range and expression in a new coelomocyte type. *Journal of Cell Science*121:339-348.

Buckley, KM & **LC Smith**. 2007. Extraordinary diversity among members of the large gene family, *185/333,* from the purple sea urchin, *Strongylocentrotus purpuratus*. *BMC Molecular Biology,* 8:68.

Graduate Environment at GW: The Department of Biological Sciences at the George Washington University is an excellent graduate student research and teaching environment, comprised of faculty studying a gamut of disciplines including comparative immunology, neurobiology, development, cell biology, genetics, bioinformatics, cell biology and many others. The Department is ideally equipped for work with comparative animal models and GW has state-of-the-art facilities for cell and molecular biology in the new Science and Engineering Hall including high throughput sequencing, a genomics core, a microscopy suite, a flow cytometry facility, and an aquatics suite. Major scientific organizations (National Science Foundation, National Institutes of Health, Howard Hughes Medical Institute including Janelia Farm) in addition to other Academic Institutions (Georgetown Univ, Univ of Maryland, American Univ, Howard Univ, George Mason Univ, etc) are located in the vicinity of the GW Foggy Bottom campus. The interdisciplinary and collaborative research community within the Department of Biological Sciences, on the GW campus and across neighboring institutions is an ideal environment for training graduate students interested in pursuing research or research-related careers. Moreover, Washington DC offers a very high quality of life, with easy access to the Smithsonian Institution, the National Zoo and USDA laboratories. Members of the Department of Biological Sciences are involved in several special graduate programs that take advantage of collaborations with other researchers, faculty, and facilities at GW and elsewhere in the Washington area.

Financial support for PhD students is available on a competitive basis. Applications for admission for the fall semester 2016 are due by January 2, 2016.

Profile of candidates: The positions are suitable for highly motivated students with BSc or MSc degrees in Biology or related field, who are interested in integrative biology, preferably with some experience in one or more of the following areas: molecular biology, genetics, immunology, comparative or invertebrate biology, and molecular virology. The research requires a multidisciplinary approach with the use of molecular, cellular and immunological techniques. Students have daily opportunities to interact with other members (faculty, postdocs, grad students and undergraduates) of both labs, in addition to members of the laboratory of Dr. Ioannis Eleftherianos, who investigate *Drosophila* immunology. Good knowledge (speaking and writing) of scientific English is required.

How to apply: Interested candidates are invited to submit their application, including a cover letter, a full *Curriculum Vita*, and a brief description of prior research experience, together with the names and addresses of one or more referee(s)**.**

Application materials can be obtained on-line from
(http://www.gwu.edu/graduate-admissions)

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