ISJ 19: 69-84, 2022 ISSN 1824-307X

REPORT OF MEETING

XXIst scientific meeting of the Italian Association of Developmental and Comparative Immunobiology (IADCI), February 16-18 2022, Didactic Pole, Department of Biology, University of Padua, Italy

Organizers: **L Ballarin, V Matozzo, F Sandrelli, G Santovito, P Venier**

Department of Biology, University of Padua, Padua, Italy

Award "Soci non strutturati" (Best presentation and curriculum studiorum for members under 35)

Characterization and functional role of a novel C1qDC from a colonial ascidian

A Peronato[1], N Franchi[2], M Tabarelli[3], L Ballarin[1]
[1]Department of Biology, University of Padua, Padua, Italy
[2]Department of Biotechnology and Life sciences, University of Modena and Reggio Emilia, Modena, Italy
[3]PhD school in Agricultural Science and Biotechnology, University of Udine, Udine, Italy

The complement system is present in all the metazoans as a complex array of soluble and membrane proteins able to orchestrate innate immune responses such as inflammation and phagocytosis. Although the complement system of invertebrates has been much less studied than that of vertebrates, however, it is equipped with at least the alternative and the lectin activation pathways.

The C1q-domain-containing (C1qDC) proteins are a large family of proteins, present in both vertebrates and invertebrates, characterized by one or more globular C1q (gC1q) domain(s) at the C-terminus. C1qDC proteins are distinguished in C1q-like proteins, with a gC1q domain and a collagen-like region at the N-terminus, and globular head C1q proteins (ghC1q) with one or more gC1q domains and a short N-terminus with no defined domains. The latter can be further divided into proteins without a signal peptide (cellular ghC1qs or cghC1qs) and proteins endowed with a signal peptide (secreted ghC1qs or sghC1qs). The gC1q domain has a typical jelly roll topology of five pairs of anti-parallel β-strands creating two β-sheets, with eight conserved hydrophobic amino acids and can interact with a large variety of ligands, both self and non-self. The same topology is present in the tumor necrosis factor (TNF) domain of protein of the TNF family so that a C1q-TNF superfamily of proteins (C1q/TNF-related proteins or CTRPs) has been defined. The mammalian complement component C1q, a subunit of the C1 complex of the classical complement activation pathway, has been the most thoroughly studied vertebrate C1qDC protein. In addition to activating C1r and C1s (and, as a consequence, in C3), C1q can also act as pattern recognition receptor (PRR) as, through its qC1q domain, it can recognize and bind pathogen-associated molecular patterns (PAMPs) on the surface of microbes and modulate their phagocytosis. Most of the C1qDC proteins have only a gC1q domain but the presence of molecules with multiple tandem C1q domains have been reported in both invertebrates and vertebrates; among the latter, CTRP4 is the only protein with two C1q domains described in mammals, birds, reptiles, amphibians and teleosts.

The compound ascidian *Botryllus schlosseri* is a chordate invertebrate that relies only on innate immunity for its defense. Immunocytes (i.e., cells with defined roles in immunity) represent the great majority of the circulating hemocytes: they include cytotoxic morula cells and phagocytes. In this same species, we identified the key components of the lectin and the alternative pathways. All these complement components (C3, Bf, MBL, ficolin and MASP), are expressed by morula cells, the most abundant circulating hemocyte.

In this study, we mined the available transcriptomes and identified, in *B. schlosseri*, a novel multidomain C1qDC protein (BsC1qDC). It belongs to the sghC1q proteins and contains two gC1q domains, a signal peptide and present high similarity with human CTRP4. We followed the expression of BsC1qDC during the colonial

blastogenetic cycle and in colonies injected with Gram (+) bacteria and identified its mRNA location by in situ hybridization (ISH). The expression trends during the colonial blastogenetic cycle suggest the presence of checkpoints modulating the transcription of bsc1qdc. The protein is synthesized and released by morula cells and a minority of phagocytes. When we knocked down the gene, we observed a decrease in phagocytosis of target particles, probably related to the involvement of BsC1qDC in the opsonization of non-self, as well as a decrease in degranulation and is involved in.

Ongoing studies are trying to better clarify the role of bsc1qdc in *Botryllus* immune modulation and its interplay with the other complement components such as complement control proteins.

Award "Giovani laureati" (Best presentation and curriculum studiorum for members under 29)

Immune contribution to tentacle regeneration in adult mollusc and cnidarian models

G Bergamini[1], S Sacchi[2], M Ahmad[1], M Cocchi[1], S Basu[3], A Ikmi[3], D Malagoli[2]
[1]Department of Chemistry and Geology, University of Modena and Reggio Emilia, Modena, Italy
[2]Department of Life Sciences, University of Modena and Reggio Emilia, Modena, Italy
[3]Developmental Biology Unit, European Molecular Biology Laboratory, Heidelberg, Germany

Adult regeneration is a fascinating process that consists in regrowth and regain of function of tissues and organs. The role and contribution of the immune system and immune-related pathways in adult vertebrate and invertebrate regeneration have been investigated for a long time, but important gaps remain. The freshwater snail *Pomacea canaliculata* and the sea anemone *Nematostella vectensis* are two phylogenetically distant organisms, with regenerative capabilities in adult life. These models present different innate immune components, and we focused on their involvement during tentacle regeneration. The two cephalic tentacles of *P. canaliculata* are sensory components used for food search, co-specific recognition and orienting. In *N. vectensis*, the numerous oral tentacles (4-18) are extensions of the diploblastic body, forming appendages that feed, defend and expand the surface area of the gastric cavity.

Histological studies focusing on the early cephalic tentacle regeneration in *P. canaliculata*, have demonstrated that wound closure and blastema formation took place within 24 h post amputation (hpa). A Matlab® plugin allowed the semi-automated identification and quantification of a phagocytic hemocyte sub-population in the blastema. Flow cytometry analysis showed that the injection of the phagocyte-specific drug Clophosome® (45 µg/g snail) could transiently remove circulating hemocytes, that recovered the pre-treatment level within 24 h. Consistently, histological experiment demonstrated that rare hemocytes were present in the early regenerating tentacles of Clophosome®-injected snails. Moreover, the hemocyte depletion impacted on regeneration time, and the blastema took twice as long to form, i.e., 24 h. This extended time overlaps with the time of recovery from Clophosome® treatment, further suggesting a role for *P. canaliculata* hemocytes in the onset of tentacle regeneration.

Differently from molluscs, *N. vectensis* presents no specialized immune cells, though cells displaying phagocytic activity were recently identified. Moreover, components of the main pathways of invertebrate humoral immunity are present. A transgenic line labelling a highly motile population of cells (mPC), similar in shape to vertebrate macrophages, enabled us to investigate their behaviours in homeostasis and regenerating conditions. Because immunostaining showed an accumulation of mPC to the wound site at 6 hpa, a high-resolution live imaging method was developed to investigate in real time to validate their direct migration to the wound site. In vivo imaging showed that mPC are positive for SoxB2, a neural precursor marker, which suggests that mPC originates from a neural cell lineage during development and differentiate in a migrating cell type. To define the molecular signature of mPC, we used FACS to sort them in view to perform RNA SMART-sequencing and characterize their gene expression.

In all, these data suggest a pivotal role for hemocytes during early stages of tentacle regeneration in *P. canaliculata*. Similarly, our original data on *N. vectensis* suggest the presence of cells imitating the behavior of molluscan hemocytes during early stages of regeneration. Intriguingly, mPC also seem to share their origin with neural cells, thus providing an example of the tight connection between immune and nervous systems also in diploblastic animals.

PLENARY LECTURE I

Immune-microbiota interplay in oyster health and disease

D Oyanedel, A Lagorce, G Charrière, M-A Travers, D Destoumieux-Garzón
IHPE, University of Montpellier, CNRS, Ifremer, Université de Perpignan Via Domitia, Montpellier, France.

The presence of complex host-associated microbial communities is a characteristic shared by most animal species and the type of interactions that they establish with their host can range from mutualistic to pathogenic. The capacity for microbes to colonize a host depends on both host and microbial determinants. In the marine environment, bivalve mollusks constitute habitats for bacteria of the Vibrionaceae family. *Vibrio* belong to the microbiota of healthy bivalves, which have the ability to concentrate bacteria in their tissues and body fluids, including the hemolymph. The oyster immune system tolerates rather high amounts of *Vibrio* in its hemolymph. However, *Vibrio* can also proliferate in oyster tissues leading to mass mortalities of oysters. Other microorganisms such as the OsHV-1 virus have the ability to alter oyster immunity leading to