REPORT OF MEETING

# XVIIIth scientific meeting of the Italian Association of Developmental and Comparative Immunobiology (IADCI), 8 - 10 February 2017, Department for Innovation in Biological, Agro-food and Forest systems (DIBAF), University of Tuscia, Viterbo, Italy

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## First evidences of a complement system lytic pathway in invertebrates: data from the compound ascidian *Botryllus schlosseri*

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The complement system is well studied in mammals, where more than 30 proteins have been described, involved in the activation and regulation of this important humoral effector. However, the evolutionary history of the complement system is not yet fully elucidated and, in recent years, it has been widely demonstrated that complement system is more than just a defender against intruders. For instance, it is important for the clearance of apoptotic cells and corpses.

*Botryllus schlosseri* is a cosmopolitan ascidian, belonging to the phylum Chordata, considered a model organism for the studies of the evolution of the immune system.

Studying the complexity of the complement system in *Botryllus,* we identified a transcript, in our EST collection, coding a protein containing the MACPF (membrane attack complex/perforin) domain, shared by both most of the proteins involved in the lytic pathway and perforins. In vertebrates, we know that perforins are produced by T-lymphocytes and natural killer cells, whereas the activity of the proteins, with the MACPF domain is regulated by the complement component C3/C5.

Comparing the domain's topology of vertebrate C9 and our protein, called *Botryllus* C9-like protein (BsC9), a high level of similarity results. To demonstrate that our C9-like protein can be considered a part of the complement system in *B. schlosseri* we evaluated the expression of BsC9 with respect to C3 activity. Our previous data demonstrates that *B. schlosseri* C3 is activated by zymosan. We combined the microinjection of zymosan with and without a validated anti-C3 antibody (against human C3) to block the activity of C3. With this approach we studied in, time course, the expression of both BsC3 and BsC9 demonstrating that the anti-C3 antibody is able to inhibit the expression of BsC9. These results are confirmed in both ISH and ICC using the same antibody, and *in vitro* with the C3 inhibitor compstatin. In addition, a significant ($p < 0.05$) decrement of labeling with BsC9 antisense riboprobe and validated antibody anti hsC9 is observed when hemocytes are incubated with zymosan and compstatin. Collectively, these results argue in favor of the presence of components of the lytic pathway in our model organisms.