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REPORT OF MEETING

XXIst scientific meeting of the Italian Association of Developmental and Comparative Immunobiology (IADCI), 12 - 14 February 2020, Department of Biotechnology and Life sciences (DBSV), University of Insubria, Varese, Italy

Organizers: **Annalisa Grimaldi, Magda de Eguileor, Gianluca Tettamanti, Roberto Valvassori, Nicolò Baranzini, Daniele Bruno, Aurora Montali, Laura Pulze**

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Session 1. Chairmen: Maria Rosaria Coscia, CNR of Naples, Naples, Italy and Piero G. Giulianini, University of Trieste, Trieste, Italy

Fish lymphocytes as an equivalent of mammalian innate-type lymphocytes?

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The immune defence system of vertebrates in its molecular and cellular components is remarkably conserved from teleost fish, the more ancient extant representatives at the base of the evolutionary lineage that brings to mammals. Multiple observations support the hypothesis that a layered system of immune responses accumulated among vertebrates over evolution, and lower layers behave as the immune system actually present in extant fish species. In this view, lymphocytes are classified as responsible of acquired responses, but recent evidences show that mammalian lymphocyte subpopulations may behave as innate cells, engaging non-self rapidly and without antigen presentation.

Innate-like lymphocytes i) maintain gut homeostasis and provide early responses to intestinal infections, ii) are involved in autoimmune diseases and cancer iii) are able to combat non-self in a MHC-independent fashion, iv) produce unbiased natural polyreactive antibodies, v) are associated to typical cytokine patterns. The main lymphocyte subpopulations displaying innate-like activities in mammals have been identified as B1-B cells, γδ-T cells, MAIT cells, and NKT cells.

Our research focuses on the spatial and temporal origin of fish lymphocyte populations, on their *in vitro* activities, and on the molecular and morphological signatures of fish lymphocytes. The aim is to predict knowledge related to mammalian innate lymphocytes from fish lymphocytes and to model human diseases. This review will present evidences suggesting the similarities between fish lymphocytes and mammalian innate-like lymphocytes.

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T lymphocytes in the sea bass (*Dicentrarchus labrax*) intestine

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The European sea bass *Dicentrarchus labrax* is a marine species in which lymphocyte distribution in the digestive tract (DT) was first described, and much knowledge on DT immuno-physiology is available, including gene expression profiling that revealed functional specialization along the DT.

lumbricin-related peptide revealed the closest relationship with lumbricins from the leech, *Hirudo medicinalis* and *Lumbricus rubellus* earthworms by phylogenetic analysis. Most prominent mRNA expression occured in the foregut (pharynx, gizzard), while other organs had fair (body wall, midgut, ovary, metanephridium, seminal vesicles, ventral nerve cord) or low (coelomocytes) levels. By means of coelomocyte sorting, only the amoebocyte subpopulation has evidenced the mRNA expression for both peptides. During embryogenesis, a gradually increasing expression pattern was observed in the various embryonic stages that were coincident with the mRNA expression pattern during tissue restoration processes. Following 48 h of *in vivo Staphylococcus aureus* bacteria challenge both mRNAs were significantly elevated in coelomocytes, while *Escherichia coli* bacteria or *zymosan* stimulation had no distinguishable effects.

Since *E. andrei* earthworms are widely applied in various toxicological assays, these novel peptides can be monitored as potential molecular targets that may be modulated by environmental contaminants.

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Characterisation of the complement system of a colonial protochordate: study of the expression of C3, CR1, C3AR and their role in nonself recognition

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The complement system is one of the most ancient immune effector mechanism of bilaterian metazoans. Three complement-activation pathways are known in vertebrates: the classical, the alternative and the lectin pathways.

In the compound ascidian *Botryllus schlosseri*, a reliable model organism for the study of immunobiology, we demonstrated the presence of the lectin and the alternative pathways. All the complement components identified so far, are expressed by morula cells, the most abundant circulating hemocytes.

In mammals, once the complement system is activated, C3 is cleaved to C3a and C3b, the former exerting a chemokine–like activity, the latter acting as opsonin and, ultimately, activating the lytic pathway. In the present work, we continued our analysis of the role of C3 in *Botryllus* immunity by studying the modulation of BsC3 transcription during the colonial blastogenetic cycle and the effect of bsc3 knockdown on immune responses.

In addition, we looked for putative complement receptors. In mammals, the best-known receptor for C3a is C3aR, whereas CR1 is the receptor, on the phagocyte surface, able to recognize and bind C3b. Here, we describe, in *B. schlosseri*, a gene showing similarity with vertebrate C3aR and three genes with similarity to CR1 (two soluble forms and one transmembrane). We also studied their transcription in the course of the colonial blastogenetic cycle. Results indicate that complement receptor mRNAs are located in different immunocytes, suggesting the presence of a cross-talk between phagocytes and morula cells. Only morula cells, and no other immunocytes type, were labelled by the antisense probe for BsC3aR and the soluble CR1s, whereas phagocytes and young, undifferentiated cells known as hemoblasts were the cells stained by the probe for the membrane-linked BsCR1. Both the *bsc3ar* and *bscr1* genes are constitutively transcribed; however, a modulation of transcription occurs during the colonial blastogenetic cycle as the amount of BsC3aR mRNA abruptly decreased at take-over, whereas no differences were observed when early-cycle and mid-cycle were compared. This is probably related to the renewing of circulating cells at TO, when 20 - 30 % of hemocytes undergo cell death by apoptosis and are replaced by new, differentiating cells entering the circulation in the same period.

Evidence for the chimeric origin of a pheromone-coding gene in *Euplotes raikovi*

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Ciliates, unique among eukaryotes, evolved two types of nucleus which are distinct in both structure and function. A diploid transcriptionally silent germline micronucleus (MIC) with an orthodox chromosomic structure coexists in the same cytoplasm with a polyploid transcriptionally active somatic macronucleus (MAC) showing a unique sub-chromosomic structure. This structure is acquired in coincidence with every sexual event, when the ex-conjugant (or ex-autogamic) cell initiates a new life cycle developing a new MAC from a mitotic product of the synkaryon. In spirotrichous ciliates such as *Euplotes*, the chromosomes of this product undergo impressive phenomena of polytenization, fragmentation in thousands of DNA fragments known as 'Macronuclear Destined Sequences' (MDSs), and DNA elimination. Under the guide of noncoding RNA templates synthesized by the old MAC before being destroyed, these MDSs are assembled into sub-chromosomic ('gene-size') DNA molecules which, amplified to thousands of copies, compose the new MAC. The way to a correct MDS assembly may be crossed by errors, with the consequent generation of functional chimeric genes which can stably be integrated and expressed in the MAC genome.

One of these chimeric genes came to light by studying the genetic basis of the pheromone-mediated self/not-self recognition mechanism in *E. raikovi*. The genome of type I cells secreting pheromone E*r*-1 was found to contain two structurally distinct MAC E*r*-1 coding genes, both expressed via a mechanism of intron splicing responsible for the synthesis of the E*r*-1 soluble

form and a membrane-bound E*r*-1 isoform functioning as autocrine pheromone receptor. The sequence of one gene resulted unmistakably homologous throughout its length with the sequences of other members of the *E. raikovi* pheromone gene family. In contrast, the sequence of the second gene resulted unique at level of a 359-bp segment of the 5' region destined to specify the cytoplasmic domain of the E*r*-1 membrane-bound isoform. By showing that this segment arises from a wrong assembly of a MDS destined to a 2417-bp gene with no relationship with the signaling pheromone system, we provide additional evidence that the generation of functionally active chimeric genes from not-programmed phenomena of somatic MDS recombination is an effective and MIC-independent source of gene variants in the ciliate MAC genome.

The Mediterranean anthozoan *Anemonia viridis* (Forsskål, 1775) for the study of inflammation and regeneration

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Regenerative capability in anthozoans is an important adaptive strategy for their survival to environmental disturbance of natural and anthropogenic origin such as predation or anchoring, that can cause injuries or removal of entire parts of the animal body, and it can be also considered indirectly a further tool of innate immune system. In the context of "self"-"non self" recognition, is significant the interaction with the endosymbiont of the genus *Symbiodinium* and the recognition of pathogens and foreign agents capable of invading the injured tissues.

From these premises and the growing stressors that can cause injuries, it is significant to understand how species respond to physical damage and how they manage to recover and regenerate compromised tissues.

Our research team, studied in the Mediterranean anthozoan *Anemonia viridis* (Forsskål, 1775), the natural seasonal variability of its morphology and enzymatic biomarkers involved in inflammatory process, the immune system response following injection of molecules varied in type and dimension. In particular, after the infection of two pathogenic bacteria *Escherichia coli* and *Vibrio alginolyticus* a particular and strong reaction was observed. These previous knowledge allowed us to examine the activity of enzymes such as proteases (SDS-PAGE on gelatin and fibrinogen substrate), peroxidase and alkaline phosphatase as biomarkers traditionally involved in wound healing event and in the rearrangement of extracellular matrix.

The regenerative process, in this Mediterranean species of anthozoan, was analyzed by subjecting groups of animals to differential tentacle cuts (n = 10, 20, 30) and observing and estimating the regenerative potential after 7, 14 and 21 days from cutting. A morphological and histological observation of the tentacular regrowth event was conducted in addition to the evaluation of the expression of proliferating cell nuclear antigen (PCNA) using the immunoblotting technique.

The future goal is to increase the knowledge of the processes that trigger the immune response in inflammation and regeneration, for the considerable interest in basic sciences and for the transferability of results in biotechnological field.

Morpho-functional characterization of the microenvironment in muscle cell development of *Hirudo verbana*

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Muscle regeneration is a process of great interest to the scientific community because is involved in a big number of disorders that are still without a cure. To study the mechanisms behind this process, the most used animal models have always been rodents, for their closeness to humans. Nonetheless, the rising ethical awareness has encouraged a reduction in the use of vertebrates in the research, but at the same time the complex puzzle in which the different factors interact for muscle regeneration has not been clarified, so the contribution of the *in vivo* experiments is still fundamental.

The leech is an inexpensive and easily manageable animal model and represents an invaluable and necessary alternative for these studies. In fact, in a simple body organization it is able to elicit complex processes (wound-healing, angiogenesis, immune response, and muscle regeneration) characterized by the same phases described for vertebrates.

For all these reasons, taking also in consideration that regeneration recapitulates some of the processes employed during development and that no satellite cells were identified in leech, we aimed to study the structure and composition of the microenvironment where myogenic precursors mature during the body wall growth of the juvenile leech.

In this regard, we performed morphological analyses of the adult and of the juvenile of *Hirudo verbana,* focusing on the extracellular matrix (ECM) organization and on the cell's level of differentiation. Moreover, we also reconstructed one of the main pathways by which mechanical stimuli are integrated and transduced into transcriptional activity: the *Hippo signaling pathway*.

Our data suggest that ECM has a pivotal role in controlling myocytes' proliferation, migration and differentiation thanks to the control exerted by Yap1. Moreover, many cellular types, which concur in this development as stem-like cells of different lineages, were identified in these processes. These preliminary data confirmed that juvenile leech can be a good model for understanding how muscle fibres can growth and differentiate in relation to the