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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

Organizers: **G Scapigliati, AM Fausto, M Mazzini, N Romano, F Buonocore, S Picchietti, MC Belardinelli**

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Session 1. Fish Immunity
Chairman: Giuseppe Scapigliati, University of Tuscia, Viterbo, Italy

Lecture
Evolution of immunity - from invertebrates to vertebrates

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Interspecific communication between various organisms in the environment is relying on a series of basic biological processes which can be found in both invertebrates and vertebrates. Even the most primitive unicellular organisms, such as amoebae, must find a balance between tolerance to surrounding elements and highly protective responses against potentially pathogenic microorganisms. The balance is well illustrated by the process of phagocytosis: this is a way for a cell to import energy and nutrition to sustain life processes but the process can also engulf and inactivate invading pathogens when combined with a range of effector molecules. These events can be supported under different environmental conditions. We presume that primitive unicellular organisms, solitary or residing in colonies, during the relatively early history of the Earth, more than 1000 MYA, occupied anaerobic sediments. This environment challenged survival of the local inhabitants which possessed advanced cellular machinery to extract energy from the substrate and at the same time were able to recognize and respond to pathogen associated molecular patterns. This early development of pathogen and danger recognition receptors is still the basis for survival in more developed animals appearing after the Cambrian explosion. Although photosynthesis and thereby oxygen placed an additional evolutionary pressure on the early organisms, and thereby gave rise to new life forms, a crucial conservation of an anaerobic environment with its associated microorganisms in the intestine of higher animals, plays a pivotal role for host immunity. Concordantly, the importance of the gut microbiota in advanced mammals, such as man, has been well documented for a number of immunologically determined diseases (e.g. diabetes and IBD). Teleosts represent one of the earliest vertebrate groups with a developed adaptive immune system comprising MHC, T-cell receptors and several classes of immunoglobulins. As a range of basic innate effector molecules are available as well, it is worthwhile to study basic immune mechanisms (both innate and adaptive) in fish. This is illustrated by the rainbow trout, *Oncorhynchus mykiss*, which can be immunized against the enterobacterium *Yersinia ruckeri* by several routes. Vaccination studies using this host species show how timing and administration of antigen (injection, immersion, bath, oral) can

Cells extracted from MG were cultured and characterized with the specific markers CD45 and CD68, confirming their belonging to the monocyte-macrophage lineage. Primary macrophage cultures were then subjected to an *in vitro* treatment with MWCNTs at different concentrations (2.5, 5, 10, 25, 50 and 100 µg/ml).

Our results indicate that leech macrophages, once in close contact with MWCNTs, actively produce amyloid material to encapsulate the foreign bodies. We also demonstrated that MWCNTs in vitro treatment cause the decrease of cell proliferation rate and the increase of the apoptotic rate. Furthermore, since oxidative stress is linked with inflammation and amyloid production, reactive oxygen species has been evaluated, confirming that their production rate increases after MWCNT treatment.

Our combined experimental approaches, not only attest the ability of MWCNTs in inducing a potent inflammatory response, but also confirm the medicinal leech as a good alternative model that can be improved and successfully used to study the possible harmful effects of any nanomaterial. Moreover, since autophagic cell death pathway activation is emerging as a possible consequence of MWCNT treatment, in the future we will attempt to clarify this aspect in order to completely understand MWCNT-induces toxicity.

The human recombinant RNASET2 activates the initial phase of the inflammatory response in the medicinal leech

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Recent studies have demonstrated that RNASET2, the only member of the ribonuclease T2 family present in human genome, is involved in the control of tumorigenicity in ovarian cancer cells. Furthermore its capacity to be a chemoattractant for numerous cells of monocytic-macrophage line and the possibility to act as an inducer of the innate immune response in Vertebrates have been established. In fact, in tumor tissues the detectable cross-talk between cancer cells and the surrounding tumor microenvironment is based on these aspects. Although several studies have been reported on the molecular features of RNASET2, the details on the pathways by which this evolutionarily conserved protein regulates the immune system are still poorly defined. In order to better elucidate these aspects, we report here the effect of the human recombinant RNASET2 injection and its role in regulating the innate immune response after bacterial challenge in an invertebrate model, the medicinal leech. This animal has been chosen for its very simple anatomy and for its rapid inflammatory response. Indeed, after few hours from the injection, a large number of fibroblasts are visible in the connective tissue that appears completely remolded and infiltrated by numerous cells expressing the specifics macrophage markers CD68 and *Hm*AIF1. In order to confirm the ability of this ribonuclease to attract macrophages, we used a consolidated experimental approach based on the injection in the leech body wall of the Matrigel biomatrice (MG), supplemented with the human recombinant RNASET2. After one week, the extracted MG sponges was infiltrated by numerous cells $CD68^+$ and *Hm*$AIF1^+$. Moreover, in the leech body wall challenged with lipopolysaccharides (LPS) or with the environmental bacteria pathogen *Micrococcus nishinomiyaensis,* endogenous RNASET2 is highly expressed by the numerous macrophages migrating to the site of inoculation. Taken together, these results clearly suggest that RNASET2 is likely involved in the initial phase of the inflammatory response in leeches.

Session 3. Invertebrate immunity
Chairmen: Davide Malagoli, University of Modena and Reggio Emilia, Modena, Italy

Genomic immune system of ciliates: DNA elimination as a genome defense mechanism

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Whole genome sequencing analyses are providing compelling evidence that pro- and eukaryote microbes, like multicellular organisms, have their life threatened by parasitic attacks. Bacteria and Archea face invading viral nucleic acids with an 'inheritable DNA-encoded immunity' (known as the CRISPR-Cas system) that recognizes foreign DNA from self DNA. In eukaryotic microbes that are exposed to invasions from both bacteria and viruses, bacteria are promptly made harmless either by digestion into food vacuoles, or by 'domestication' as symbionts. But the defence from viral attacks is much less effective. Foreign viral sequences can randomly insert into the cell genome, and may disrupt or deactivate vital genes.

To fight this threat, ciliates rely on a unique model of inheritable genomic immune mechanism based on the evolution of two genomes, a germ-line one lying in the cell micronucleus and a somatic one lying in the macronucleus. The germ-line genome characterized by an orthodox chromosomic organization exposed to invasive viral DNA sequences is maintained transcriptionally silent. Only the somatic genome characterized by a unique sub-chromosomic organization is expressed. It is generated de-novo in

coincidence with every sexual event from a copy of the micronuclear genome that is previously made free of any invasive DNA sequence by the activity of a small RNA-targeted DNA-deletion mechanism, called 'Internal Eliminated Sequences (IES)-associated gene system' by ciliatologists. The discovery, function and effects of this mechanism that eliminates invasive DNAs from the developing somatic genome will be the object of this contribution.

Assessment of morphological and cellular responses after infection with living bacteria in the marine mussel *Mytilus galloprovincialis*

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Bacterial strains of *Vibrio* genus associated with temperate regions are linked to mussels-borne infections. The sedentary nature of marine mussels, *Mytilus galloprovincialis*, together with their filter feeding combine to ensure that they have the potential for considerable exposure to infective agents.

The primary mechanism of bivalve internal defense involves hemocytes responsible for cell-mediated immunity through a panel of activities such as phagocytosis, the release of cytotoxic molecules, reactive oxygen intermediates, lysosomal enzymes, PO enzyme and lysozyme.

In this work *in vivo* infection of *M. galloprovincialis* with living *V. splendidus* to collect hemolymph from the posterior adductor muscle 1, 3, 6, 9, 12, 24 and 48 h post-injection was carried out.

Previously we have found that when bacteria were injected into the circulation of the mussel, the number of living intra-hemocyte bacteria dramatically increased already after thirty minutes, suggesting intense phagocytosis, then decreasing until 24 h. The quantification by flow cytometry indicated a variation of proportions of the three cell categories.

In our study, injection of living bacteria resulted in total hemocyte count (THC) higher than normal 24 - 48 h post-injection, suggesting proliferation and/or recruitment of hemocytes which are mainly concentrated in the site of infection.

To compliment this, here histological and immunohistochemical assessment was performed using adductor musclein order to evaluate the morphological features and cellular response spost bacterial infection. The morphological analysis showed changes in tissue organization, with an altered cell volume and recruitment of hemocytes among fibers.

The change of osmotic equilibrium across muscle cell membranes was observed by increased the staining of Na-K ATPase during the entire period of stimulation, and reduced immunopositivity of aquaporin (AQP) 1 h post infection but with an increasing trend in the all experimental steps.

The investigation on cellular turnover showed a tendency to recover a regular tissue structure, as highlighted by an intense immunopositivity of proliferating cell nuclear antigen (PCNA) from 24 h to 48 h post injection, as well as cell surface death receptor (FAS) and the cysteine-aspartic acid protease (CASP3) untilto 72 h post bacterial injection.

Thus, a detailed overview of the morphological and cellular responses in the mussel adductor muscle following infection with living bacteria was provided herein.

Involvement of exosomes in immune responses

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Exosomes are small-secreted microvesicles interacting with surrounding cells and implicated in intercellular communication. Besides their signaling function, these microvesicles also serve as a mechanism to dispose obsolete cellular material.

Exosomes transport proteins, lipids and nucleic acids in the form of miRNA and mRNA moreover, bearing cytokine or death receptors, they can bind to specific ligand of cells and induce signal transduction. Every tissue fluid analyzed so far (milk, saliva, tears, urine, blood, etc.) has revealed the presence of exosomes and the range of organisms that release small vesicles, including exosomes, covers almost all known life forms. Depending on the tissue of origin, they can regulate a large number of processes as brain development and function, tumor invasiveness and immune system function.

About this last issue, it is already accepted that exosomes play a crucial role in host-pathogen interactions being produced during viral, parasitic, fungal and bacterial infections and could either promote or inhibit host immunity. Just as exosomes can become agents of dissemination, they may otherwise act as agents of control within the body when unaffected. Vesicles secreted by infected cells contain substantial amounts of pathogen molecules, which are sufficient to induce modifications in