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REPORT OF MEETING

XXth scientific meeting of the Italian Association of Developmental and Comparative Immunobiology (IADCI), 13 - 15 February 2019, Department of Biology, Ecology and Life Sciences, University of Calabria, Rende, Italy

Organizers: **A Giglio[1], P Brandmayr[1], F Talarico[1], A Mazzei[1], S Marsico[2], A Naccarato[3], F Cavaliere[1], ML Vommaro[1], MC Granieri[1]**

[1]Department of Biology, Ecology and Life Sciences, University of Calabria, Rende, Italy
[2]Department of Pharmacy, Heath and Nutritional Sciences
[3]CNR-Institute of Atmospheric Pollution Research, Rende

I met you when I was very young,
at the beginning of my professional life,
and I immediately understood
that you were reserving many surprises;
I followed you in silence
when your secrets have been revealed;
how do you always be the same and always different,
the "God problem" revealed.
I admired your anatomy,
simple, elegant,
based on harmonic couplings of a wonderful domain,
classic like a Doric temple;
I admired the flexibility of your body:
the movements of the hips and elbows,
your fingers that shape the socket.
I was entranced by the dynamics of your dance.

Then I had the pleasure
to be the only one to own you in the frost of polar ice,
where you appeared to me alone,
only wonder, generated by an evolutionary miracle.

It is true that I have been unfaithful to you;
I was attracted to other molecules,
from the aesthetics of the baroque forms of the Toll-like Receptors,
from the molecular labyrinth of the third factor of the Complement,
that is leafed in luminous fragments up to the small,
but multipotent, anaphylatoxin.

Now I will not see the many sure marvels
that you reserve to those
who will follow you in the future
but I have the memory
to have loved the most beautiful of the molecules:
Immunoglobulin.

By Umberto Oreste, past Researcher at Institute of Protein Biochemistry, National Council of Research, Naples (Italy)

SELENOT transcripts (gfSelT1a, gfSelT1b and gfSelT2) have been detected. This study aimed to evaluate the cardiac expression of SELENOT, and its putative role as an endocrine/paracrine/autocrine modulator of the goldfish heart function under both normoxic and hypoxic conditions. By western blot and immunofluorescence we found that SELENOT is expressed in the heart, and the expression is increased under hypoxia. On *ex vivo* isolated and perfused goldfish heart preparations, under normoxia, exogenous PSELT, a SELENOT-derived peptide, dose-dependently enhanced myocardial contractility by involving a modulation of membrane and sarcoplasmic calcium, and a cAMP-dependent signaling. Under hypoxia, PSELT unaffected goldfish myocardial contractility but reduced myocardial nitrosative stress. These data propose SELENOT as an evolutionary conserved protein with a protective potential against cardiac hypoxia-dependent redox unbalance. They also pave the way to explore the role of the protein as a cardiac endocrine modulator in fish and a humoral intermediary of the communication between the heart and distal tissues and cells.

Session 3. Chairmen: Luigi Abelli, University of Ferrara, Ferrara, Italy and Giuseppe Scapigliati, University of Tuscia, Viterbo, Italy
Fish immunity

Immunity in the teleost digestive tract

S Picchietti
Department for Innovation in Biological, Agrofood and Forest Systems, University of Tuscia, Viterbo, Italy

Teleost fish constitute the most abundant vertebrate group, exhibiting a high assortment of morphological variations of their gastrointestinal (GI) tract related to phylogeny, ontogeny, environment and feeding habits of each species. This variability is closely linked with several specialized functions, since the GI tract is involved not only in nutrient absorption and digestion, but also in water and electrolyte balance, and immunity. Teleosts moreover, possess a complex gut microbiota, that shapes every physiological system of the host and even effects the intestinal morphology. Because the GI tract is a main route for pathogen entry, its mucosa plays an effective defence against potentially harmful determinants of the environment, while it tolerates a diversity of harmless microbes and dietary antigens. The mechanisms allowing antigen uptake over the epithelial barrier play a crucial role for maintaining the gut homeostasis and regulate appropriate immune responses, and involve the presence of antigen sampling cells equivalent to mammalian microfold and dendritic cells. Teleosts possess an extensive system for immune activation, and responses to antigen uptake have usually been reported higher after anal than oral delivery. Gene expression profiling revealed functional specialization along the GI tract, and demonstrated that the establishment of mucosal immune responses is especially relevant in the posterior intestine. Generally speaking, teleost fish do not contain lymphoid aggregates in the mucosa, however their gut harbors high numbers of diffuse T ($CD8^+$ and $CD4^+$) and B cells. IgT^+ B cells outnumber the IgM^+ cells in teleost gut and the IgsT produced display a preponderant and specialized role in mucosal immunity, being the key players in the defence against pathogens. Importantly, commensal bacteria shape B cells and Igs responses and in a parallel way, mucosal Igs and secretory component allow the host to sculpt its microbial communities. Also T cells, mostly those bearing γδT receptors, play an essential role in intestinal cell-mediated immunity and it seems they are important in tolerance or attack against the microbiota. Studies evidenced that fish gut microbiota responds to dietary manipulations, and although the interplay between nutrition and immune system is well recognised, understanding the link between diet, gut microbiota and health in fish is only at the beginning.

Evolution of immune responses: similarities between fish lymphocytes and mammalian innate-like lymphocytes

G Scapigliati, S Picchietti
Department for Innovation in Biological, Agrofood and Forest Systems, University of Tuscia, Viterbo, Italy

Innate lymphoid cells (ILCs) of Vertebrates are a cluster of innate immune cells that are classified in three groups for the expression of defined transcription factors, functional characteristics, and phenotype. The lymphocytes of Vertebrates are classically described as the cells responsible of adaptive responses, but experimental evidence suggest that subpopulations of mammalian lymphocytes may behave as ILCs, engaging non-self rapidly and without antigen restriction. The innate-like lymphocyte subpopulations have been mainly identified as γδ-T cells, mucosal associated invariant T cells (MAIT), and B1-B cells, are principally located in mucosal tissues, may be involved in human pathologies and their functions and tissue(s) of origin are not fully understood. The similarities in the morphology and immunobiology of immune system between fish and mammals have been established, but the homologies between fish lymphocytes and mammalian innate-like lymphocytes is an issue poorly considered in comparative immunology. Increasing experimental evidence suggests that main fish lymphocyte populations could have developmental, morphological, and functional features in common with innate-like lymphocytes of mammals. However, despite these similarities and with the hypothesis that mammalian innate-like lymphocytes could be evolutionarily related to fish lymphocytes, information on possible links between γδ-T lymphocytes and and B1-B cells of fish and mammals is missing, Our research is currently aimed to investigate these possible similarities in lymphocyte evolution.

Effects on immunity of exposure to microplastics in adult zebrafish

A Mancia[1], L Abelli[1], A Benkhalqui[1], C Bertolucci[1], MC Fossi[2], G Limonta[2], C Panti[2]
[1]Department of Life Sciences and Biotechnology, University of Ferrara, Ferrara, Italy
[2]Department of Physical Sciences, Earth and Environment, University of Siena, Siena, Italy

It is now widely accepted that microplastics (MPs) represent a serious concern for aquatic environments, therefore assesment of biological pathways affected is crucially relevant.

This study focused on variations of liver transcriptome, histology of gastrointestinal tract and gills, and locomotor activity of exposed fish along various days after treatment.

Adult zebrafish (3 groups, N=12 each) were fed for 20 days with dry food alone (controls) or supplemented with a mix of pristine high-density polyethylene and polystyrene microplastics (0.1 or 1 mg/L), ranging in size from <25 to 90 µm.

The exposure to MPs resulted in differential transcription of 324 genes in total, already affected at the lower dose, mainly involved in cholesterol biosynthesis (fatty acid degradation) and immunity pathways.

Up-regulation of transcripts subserving response to extra-cellular antigens, and down-regulation of others involved in innate antimicrobial response, antiviral defense and maintenance of epithelial integrity highligted defective control of pathogen entry at epithelial barriers, confirmed by occurrence of histopathological signs in both intestine and gills. Furthermore, variations in energy utilization likely accounted also for alteration of circadian rhythm of locomotor activity.

Immunodetection of IgM, IgT and pIgR in mucosal tissues of Antarctic teleost

A Ametrano[1], A Mancia[2], S Ballarin[2], A Benkhalqui[2], G Calderoni[2], G Pavani[2], MR Coscia[1], L Abelli[2]
[1]Institute of Protein Biochemistry, CNR, Naples, Italy
[2]Department of Life Sciences and Biotechnology, University of Ferrara, Ferrara, Italy

We have previously investigated the immune response at hepato-biliary level in the Antarctic teleost *Trematomus bermacchii*, a species belonging to the Perciform suborder Notothenoidei, the most abundant component of the fish fauna living in the Antarctic ocean. By that time only the IgM isotype was known and well characterized at molecular and biochemical levels in Antarctic fish.

Over the past few years we have cloned and sequenced genes encoding other two key molecules of the mucosal immune system, IgT and polymeric Ig receptor (pIgR) of T. *bernacchii*. The present study aimed at investigating the localization in mucosal tissues of IgM, IgT and pIgR in an attempt to clarify the protein occurrence and transepithelial transport. Biochemical and immunohistochemical data provided convergent data about specific mechanisms operating apical release of IgT in exocrine way, as well as depicting peculiar (maybe ancestral) features compared with well-known mechanisms described for polymeric Igs transport in mammalian tissues.

F-type lectin from serum of *Trematomus bernacchii* (Boulenger, 1902): purification, characterization and bacterial agglutinating activity.

M Dara[1], P Giulianini[2], C Manfrin[2], MG Parisi[1], M Cammarata[1]
[1]Marine Immunobiology laboratory, Department of Earth and Marine Sciences, University of Palermo, Palermo, Italy
[2]Department of Life Sciences University of Trieste, Building Q - room 306 Trieste, Italy

Lectins belong to a protein family, present in almost all living organisms and involved in several biological processes, including immune responses. Peculiarity of these proteins is the ability to bind carbohydrates due to their carbohydrate-recognition domains (CRDs).

In fish, C lectin, F binding lectin (FBL), galectin, Rhamnose-binding lectin (RBL) and pentraxin have been identified in both cartilaginous and bony fish. In addition, selectins and other genes have been found in the currently available fish genomes.

The FBL, known as fucolectins, constitute the most recent lectin family identified and structurally characterized in teleosts. The FBL family is constituted by a large number of proteins exhibiting multiples of the F-type motif, either tandemly arrayed or in mosaic combinations with other domains.

In the present study, a FBL has been purified and characterized from serum of the Antarctic fish *Trematomus bernacchii* by affinity chromatography on fucose-agarose column. Assay of inhibition from carbohydrates in fact showed affinity of this lectin for the fucose. A convincing Hemoagglutinating activity (HA) was detected towards rabbits red blood cells (RBC) and at lesser extent towards sheep erythrocytes.

The HA activity was analyzed at different temperatures. It was maintained at temperature values comprised between 4 °C and 37 °C and was completely depleted after exposure at 50 °C. In SDS-PAGE analysis, the FBL exhibited an apparent Molecular weight of 30 kDa in non-reducing conditions and an increase to 32 kDa after reduction. This difference is recognized as a classical shrinkage of F-lectins, due to the present of internal disulfide bridges.

The F lectin present on the *T. bernacchii* transcriptome show a very similar and congruent structure with a theoretical Mw 32.16 kDa and an isoelectropoint of 5.21.

Bacterial agglutinant activity (BA) of serum and purified fractions was tested towards *E. coli*. The serum showed high activity after incubation at room temperature (18 °C), as well as in the fractions. The sequence, structure, sugars specificity, fucose inhibition, molecular weight, protein shrinkage and activity against bacteria collocate this molecule on F-lectin family and thus suggesting its involvement in host pathogen interactions.