ISJ 15: 116-130, 2018 ISSN 1824-307X

REPORT OF MEETING

XIXth scientific meeting of the Italian Association of Developmental and Comparative Immunobiology (IADCI), 7 - 9 February 2018, Department of Earth, Environment and Life Sciences (DISTAV), University of Genoa, Genoa, Italy

Organizers: **L Canesi, T Balbi, M Auguste, E Grasselli, L Vergani, I Demori, R Fabbri, M Montagna, A Voci**

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Nanoparticles and the immune system

Evolution of innate immunity, lessons learned for assessing safety and efficacy of nanomaterials and nanodrugs

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Innate immune defensive mechanisms are the major surveillance and effector mechanisms deputed to maintaining the integrity and functionality of a living organisms. From plants to human beings, innate immunity has conserved a number of defensive cells, molecules and pathways. Innate immunity is the only type of immunity present in plants and invertebrates, at variance with higher vertebrates that also display slower but more specific adaptive immune functions. Immunological memory, a feature though to characterise adaptive immunity, is clearly present in innate immunity although based on different mechanisms, and can be clearly displayed by phagocytic cells, which are rapid and efficient cells in recognising and destroying invading microorganisms or foreign particles. We have studied both innate immune responses and the development of innate memory in human blood monocytes *in vitro* in comparison with the marine tunicate *Ciona intestinalis*, in which adaptive immunity is absent. The strong primary response to a bacterial challenge (*e.g.*, LPS) can prime cells *in vitro* or animals *in vivo* to respond differently to a subsequent challenge with the same or with a different stimulus. Human cells primed with LPS showed a significant modulation of their activation capacity in response to a second challenge (with LPS or other stimuli), with a clear decrease in the capacity of producing TNFα. On the other hand, challenge with LPS strongly increased the phagocytic ability of *C. intestinalis* haemocytes primed with either LPS or LTA, whereas challenge with LTA decreased it. For human cells, strong donor-to-donor variations were evident, suggesting that previous *in vivo* exposure to diverse external agents may change the reactivity of blood monocytes to *in vitro* stimuli. Although significant quantitative inter-individual differences were noted, in *C. intestinalis* the development of innate memory was more reproducible in terms of increase or decrease of secondary response, suggesting a limited or similar influence of the previous life conditions.

The comparative study of innate memory is expected to generate information that will help us controlling and modulating memory development in

increased monocyte production of CCL22 (a chemotactic factor for Treg) and inhibition of antigen-specific T cell proliferation. Hence, this study unveils unprecedented biologic functions of cDNA that may have pathogenic relevance in cancer.

Impact of marine contaminants of emerging concern on the cetacean transcriptome

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Contaminants of emerging concern (CECs) are widely distributed in the environment, but their occurrence and potential toxicity are only now being evaluated. CECs are increasingly being detected in the waters and many act as endocrine disruptor compounds (EDCs), causing a variety of effects on health. The worldwide distributed perfluorooctanoic acid (PFOA) and bisphenol A (BPA) are CECs falling in the EDCs category.

Skin samples from the bottlenose dolphin (*Tursiops truncatus*), a top predator that spends its entire life in the water and therefore subject to accumulation and magnification of contaminants, were collected to analyze the impact that environmentally relevant concentrations of CECs may have on global gene expression. We combined transcriptomic analysis and *ex vivo* assays using small skin slices cultured and treated for 24 h with PFOA or BPA or vehicle.

RNA from dolphin biopsies was labeled and hybridized to a species-specific oligomicroarray. The skin transcriptome displayed changes related to contaminant exposure, potentially predictive about long-term effects on health, being the genes affected involved in immune modulation, response to stress, lipid homeostasis, and development. Within the genes differentially expressed in the transcriptome after CECs treatment, 4 were tested as potential gene markers of anthropogenic contaminants exposure on skin samples from wild cetaceans. RNA from 12 individuals, including the species *Stenella coeruleoalba*, *T. truncatus*, and *Grampus griseus* were sampled in 3 areas (Adriatic, Ionian and Tyrrhenian seas). Three out of the 4 genes tested showed higher expression in the samples collected from the Adriatic sea.

The transcriptomic signature of a dolphin skin could be relevant as classifier for a specific contaminant whilst giving information of the specific geographic location where the marine mammal spent its life, due to the different impact on gene expression exerted by different contamination levels.

Amphibian peptides for skin protection and healing

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BACKGROUND: Amphibians are currently suffering a dramatic decline worldwide, mainly due to chytridiomycosis, a skin infection caused by the pathogenic fungus *Batrachochytrium dendrobatidis* (Bd). An important natural defense of amphibian skin is the production of antimicrobial peptides (AMPs) by granular glands in the dermis. AMPs collected from several species of frogs successfully inhibit the growth of Bd *in vitro*. Besides their anti-microbial and anti-fungal activities, AMPs have been shown to exert other biological effects such as anti-viral, anti-tumor, anti-oxidant, immunomodulating and wound healing.

AIM: We intended to test the efficacy of AMPs as cutaneous defenses in frog species either resistant or susceptible to Bd.

METHODS: 3 frog species, *Gastrotheca nebulanastes* (GN), *G. excubitor* (GE) and *Hypsiboas gladiator* (HG), were collected in montane scrub, cloud forest and high elevation grassland habitats near Manu National Park in southeastern Peru. AMP secretion was stimulated by injection of norepinephrine into the dorsal lymph sacks. AMPs were then purified by chromatographic techniques. The human endothelial cell line HECV was treated with AMP concentrations ranging from 0.005 to 50 µg/mL. Cell viability was verified by MTT test. Wound healing properties were analyzed by scratch wound assay. AMP inhibition strength against Bd growth was measured *in vitro* by incubating Bd zoospores with different concentrations of AMPs.

RESULTS: Treatment with AMPs secreted from GN, GE and HG did not affect HECV cell viability at any concentration tested. No significant differences in cell migration rate were observed in HECV cells scratched and treated with GN and GE AMPs. Only HG peptides showed wound healing properties as well as strong Bd growth inhibiting ability.

CONCLUSIONS: Stimulation of wound healing mechanisms and inhibition of Bd growth by skin AMPs might both contribute to HG resistance to chytridiomycosis. Understanding the role of skin defenses may lead to the development of novel Bd mitigation strategies. Possible applications of amphibian AMPs in skin medicine deserve attention and further studies.

Characterization of CD3ε+ T lymphocytes of sea bass *Dicentrarchus labrax L*.

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CD3 is an important cell surface marker of T