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LIBRO degli ABSTRACT

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and binding reduced PEBP1 and ERK1/2 phosphorylation as well as Ros, Nox4 and IL-8 production in CSE-treated cells. Tiotropium or Olodaterol reduce the levels of Ros, NoX4, IL-8 and ACh expression. CSE pre-treatment decrease OLO-induced cAMP release to a greater degree than prior TIO treatment. Stimulation of cells with TIO and OLO in combination for 10 minutes induced a high level of cAMP release.

Cigarette smoke impairs Sirt1 activity and promotes pro-inflammatory responses in bronchial epithelial cells

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Cigarette smoke is the major risk factor for chronic obstructive pulmonary disease (COPD), a disease where inflammation and aging are intertwined. Sirtuin (Sirt)1 is an anti-ageing factors that removes acetyl moieties and activates FoxO3, a transcriptional factor which controls cell cycle progression, cell death and inflammation and protects the cell from oxidative stress. In the present study we investigated the relationship between these anti-aging factors and inflammatory processes (NF-κB, IL-8 and CCL20 expression) in response to cigarette smoke. IL-8 and CCL20 were selected as markers for innate and adaptive responses. 16HBE cells and primary bronchial epithelial cells isolated from COPD patients and healthy controls, pre-treated with/without the Sirt1 inhibitor, Sirtinol, were stimulated with increasing concentrations of cigarette smoke extracts (CSE). The nuclear accumulation of Sirt1, FoxO3 and NF-κB, deacetylase activity of Sirt1 and IL-8 and CCL20 expression (protein and mRNA) were evaluated. The obtained results showed that (i) CSE decreases the activity and nuclear levels of Sirt1 in 16HBE cells; (ii) CSE reduces FoxO3 in 16HBE and in primary bronchial epithelial cells from healthy subjects; (iii) the constitutive expression of FoxO3 was more down-regulated in primary bronchial epithelial cells from COPD subjects than from healthy controls; (iv) CSE increased NF-κB and IL-8 expression and decreased CCL20 expression. Pretreatment with Sirtinol reduces FoxO3 and increased NF-κB and IL-8 expression but it had no effect on CCL20 expression. These data suggest that cigarette smoke impairs the function of Sirt1 leading to deregulation of FoxO3 and NF-κB activity, modifying the cellular ageing and inflammatory processes with a prevalent activity of innate immune responses.

Inflammatory reaction and isolation of multifunctional bioactive molecules in cnidarians: from Immunobiology to Blue Biotechnology

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The phylum of Cnidaria is one of the first branches in the tree of animal life to provide crucial insights on the evolution of immunity. Cnidarians are diblastic aquatic animals with radial symmetry and they are the simplest multicellular organisms that have reached the level of tissue organization. The renewed interest in the study of immunity in Cnidaria has led to additional information to the scenario of the first stages of immunity evolution revealing the cellular processes involved in symbiosis, in the regulation of homeostasis and in the fight against infections. We investigated the inflammatory response in Cnidarian following injection of various substances different in type and dimension, and observed clear, strong and specific reactions especially after injection of bacteria. The enzymes evaluation (protease, phosphatase and esterase), showing how the injection of different bacterial strains alters the expression of these enzymes suggesting a correlation between the appearance of the inflammatory reaction and the modification of enzymatic

activities. The Cnidaria phylum has evolved using biotoxins as defense or predation mechanisms for ensure survival in hostile and competitive environments such as the seas and oceans indeed the tissues and the mucus produced by cnidarians are involved in immune defense and contain a large variety of toxins such enzymes, potent pore forming toxins, and neurotoxins. They could also take advantage of the multi-functionality of some of their toxins. The bioactive molecules were characterized and purified by biological assays, acid extraction, HPLC purifications, mass spectroscopy and peptide synthesis. Here, we show the cnidarian bioactive molecules as antimicrobial peptides and enzymes in order to draw important applications in fields ranging from pharmacology to cultural heritage.

Characterization of immunomodulatory activities of Ci8short peptide induced by LPS from Ciona intestinalis

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The dysregulation of the immune response plays an important role in many diseases. Therefore the study of different cell types with immunomodulatory activities is critical for innovative therapeutic approaches. We recently isolated (Vizzini et al. 2013) a LPS-induced peptide (Ci8short) from the ascidian *Ciona intestinalis*. This peptide shows a peculiar amino acids composition suggesting the possibility that it can act as an Host Defense Peptide. For this reason, the immunological properties of the Ci8short peptide were studied by using human PBMC from healthy subjects *in vitro*. As first result, we were able to demonstrate that this peptide did not show cytotoxic or/and hemophilic activities in *vitro*. Furthermore, we observed that the Ci8short displays some immune activities showing the ability to preferentially induce the proliferation of human $CD4^+$ cells at 7 days. Following this line of evidence, we decided to perform a time course looking at the appearance of $CD4^+/CD25^+$ cells after Ci8short stimulation demonstrating that this peptide was able to select peptide-specific effector cells. In particular we demonstrated that Ci8short induces the secretion of IFN-γ, IL-10 and IL-17 cytokines by human $CD4^+$ cells. To further characterize $CD4^+/CD25^+$ cells, we evaluated the expression of an immunophenotypical marker such as the CD127 and the presence of the GARP (Glycoprotein A Repetitions Predominant), LAP (Latency-Associated Peptide) and CD39 functional markers. From this analysis, we showed that the Ci8short peptide induces the selection of $CD4^+/GARP^+/LAP^+/CD39^+$ at 7 days. The results obtained in this study will be useful for the understanding the Ci8short peptide mediated immunological mechanisms as it may have potential to be developed as a novel immune regulatory adjuvant.

Isolation of a novel LPS-induced component of the ML superfamily in *Ciona intestinalis*

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The ML (MD-2-related Lipid-recognition) superfamily contains a large set of genes encoding proteins such as MD-1, MD-2, NiemannPick type C2 (NPC2) protein, the GM2 activator protein and the mite allergen Der p 2. Members of the ML domain play important role in lipid metabolism (sterol homeostasis and steroid biosynthesis) but also in innate immune signal pathways (LPS induced signalling). In invertebrates, a few ML genes have been identified in Drosophila melanogaster (Shi et al., 2012), in the shrimp Litopenaeus