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CELL MEDIATED IMMUNE RESPONSE OF THE MEDITERRANEAN URCHIN *Paracentrotus lividus* TO PAMPs STIMULATION

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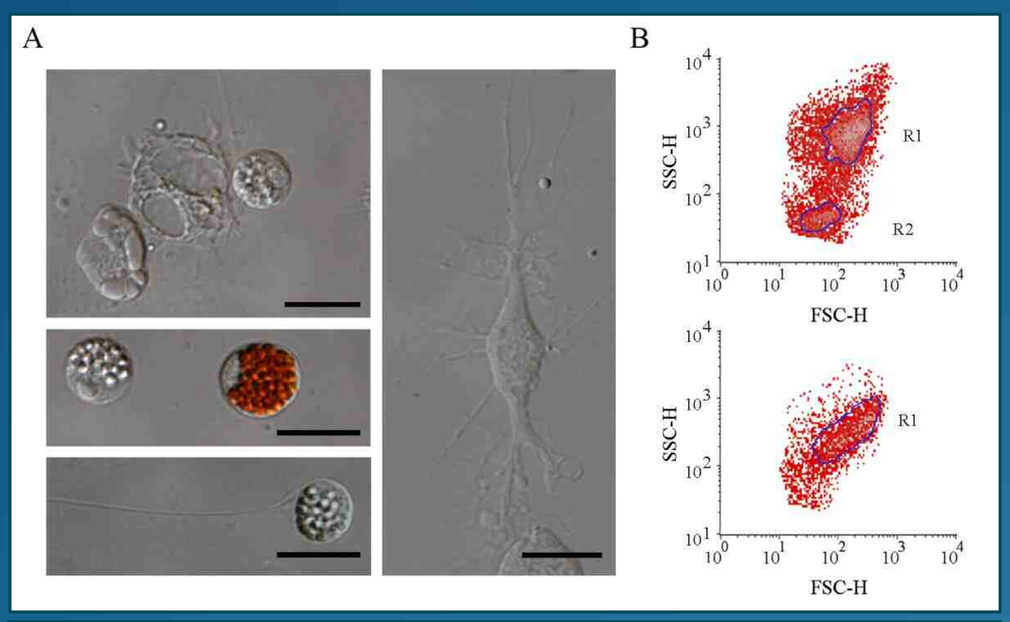
INTRODUCTION AND OBJECTIVES :

The Mediterranean sea urchin (*Paracentrotus lividus*) is of great ecological and economic importance for the European aquaculture. Few studies explain how this animal interacts with pathogens and which immune mechanisms are induced to overcome the diseases.

The immune system involves humoral and cellular components. The immune cells are coelomocytes and move in the coelomic spaces. There is not a single standard classification of coelomocytes for all echinoderms since they are heterogeneous in morphology and size. The immune functions of each type of coelomocyte are still not totally understood, but it is postulated that amoeboid-phagocytic cells and spherule cells are the only cellular components of the immune system.

In the present work some cellular immune responses were explored in *P. lividus*. **Flow cytometry** was used to evaluate the **cell cooperation, phagocytic activity** and the **production of ROS and NO** after stimulation with different PAMPs.

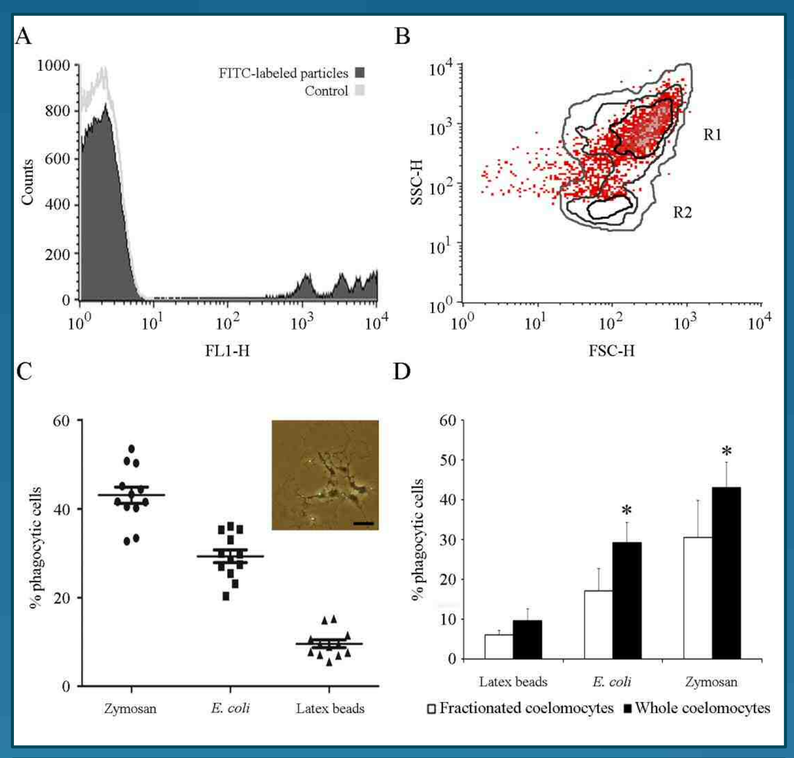
Two cell-mediated immune genes were characterized and their regulation after PAMP stimulation analyzed: A macrophage migration inhibitory factor (**MIF**) gene that regulates the inflammatory functions of immune cells and the LPS-induced TNF-α factor gene (**LITAF**) that regulates the expression of TNF-α and various inflammatory cytokines in response to LPS.



CELL TYPES AND FLOW CYTOMETRY

(A) **Four** main types: **Petaloid cells** suffering morphological transformations when spread on a glass substratum, Two classes of **spherule cells** (red or colourless) and the **vibratile cells** moving through the fluid by a long single flagellum.

(B) By **flow cytometry** two main cell populations were distinguished. **R1 and R2** represented a 63% and a 32% of the total cell population, respectively. When coelomocytes were allowed to settle and the non-adherent cells were removed, the number of cells in the R1 region increased suggesting the presence of amoeboid-phagocytes within the R1 region



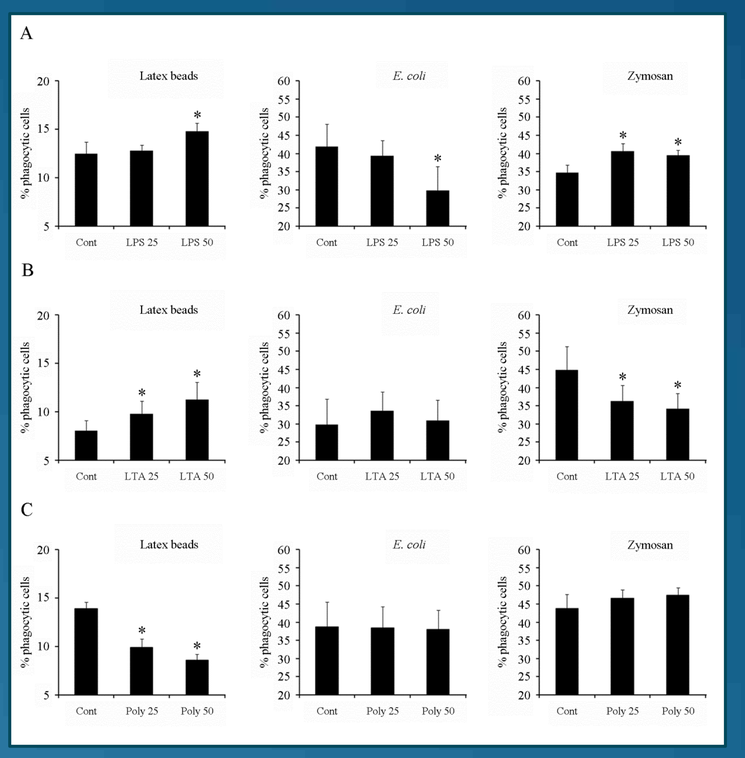
PHAGOCYTOSIS AND CELL COOPERATION

(A) The phagocytic activity was registered as an **increment in the FL1-H** fluorescence levels.

(B) The **phagocytes** are included in the **region R1**.

(C) Phagocytic coelomocytes were able to **ingest latex beads, *E. coli* and zymosan particles**. *E. coli* and zymosan particles were significantly more ingested than latex beads.

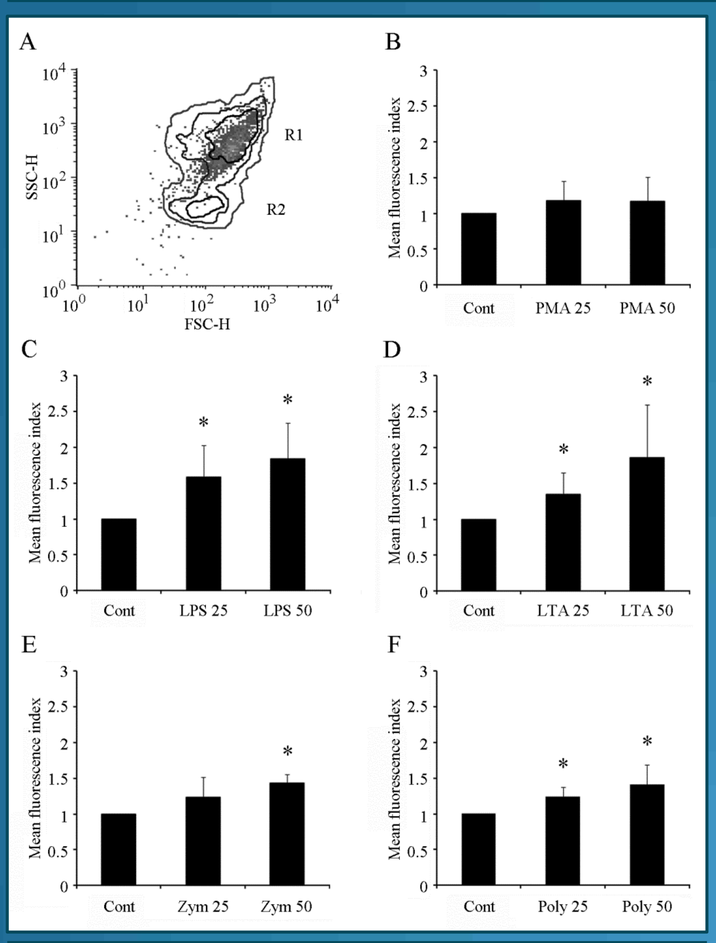
(D) **Cell cooperation:** the phagocytic rates of samples using whole coelomocyte preparations were significantly **higher** than those obtained in samples enriched in adherent cells (fractionated coelomocytes).



MODULATION OF THE PHAGOCYTIC ACTIVITY

Cells treated with **LPS and LTA** showed a significant **increment** in the uptake of latex beads and zymosan particles.

The ingestion of *E. coli*, zymosan and latex beads were significantly reduced after treatment with LPS, LTA and Poly I:C, respectively suggesting a possible **competence for the surface receptors between the stimuli and the particles.**



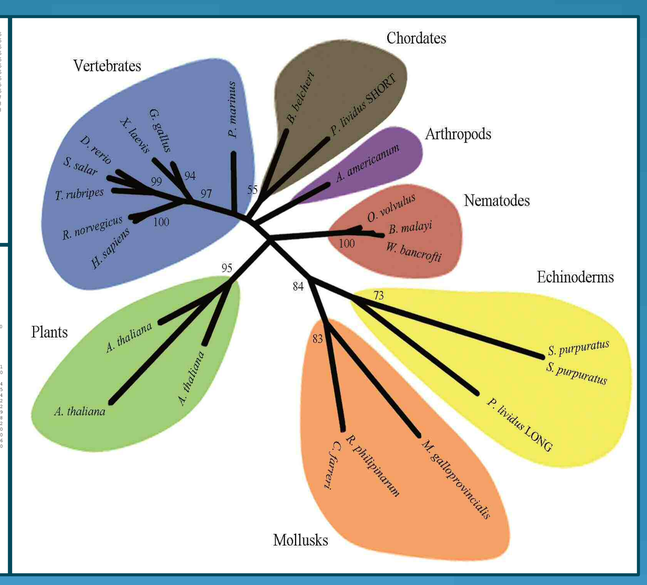
ROS AND NO PRODUCTION

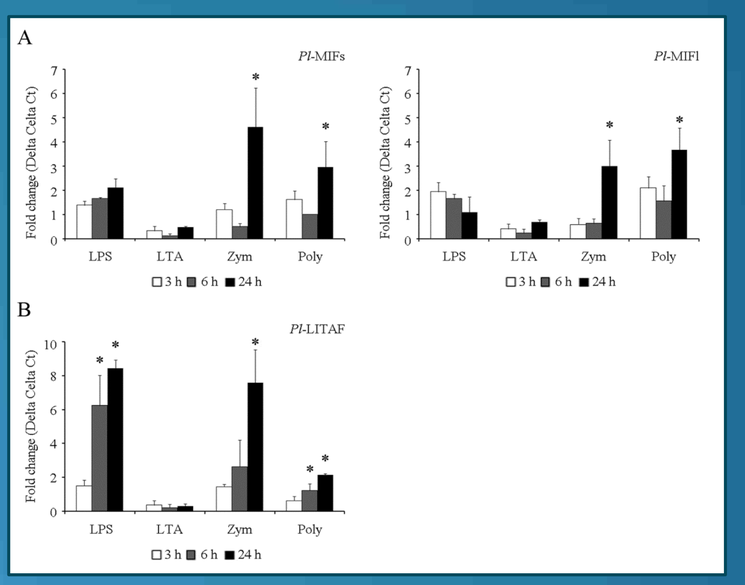
(A) **Adherent amoeboid-phagocytes** (R1 region) are the most active ROS producer.

(B) The soluble PMA did not induce any effect on the ROS production

(C-F) The ROS production **significantly increased** in presence of LPS and LTA. Zym and Poly I:C induced a significant but lower production of ROS.

Coelomocytes maintained a **low basal level of Nitric Oxide** not being modified after treatment with PAMPs (data now shown)





STRUCTURAL AND PHYLOGENETIC CHARACTERIZATION OF MIF AND LITAF GENES

Two cell mediated immune genes were selected from the **public *P. lividus* EST database** (http://goblet.molgen.mpg.de/cgi-bin/webapps/paracentrotus2008.cgi): A macrophage migration inhibitory factor (MIF) gene and the LPS-induced TNF-α factor (LITAF) gene.

The **MIF genes** showed similar structural features than those found in other vertebrates and invertebrates. The amino-terminal proline residue (P2) which is crucial for the catalytic activity, the site of isomerase activity (K33) and two out three sites of oxido-reductase activity (C57 and C60) are conserved. The prediction of secondary structure showed two alpha-helices and five beta-sheets.

The **LITAF gene** showed the characteristic domains: a N-terminal CXXC motif, followed by a 25 residues hydrophobic region and a C-terminal (H)XCXXC motif. There were eight conserved Cys residues in the LITAF family domain.

The **branching pattern** of MIFs and LITAF corresponded essentially with the evolutionary relationship among the species.

MODULATION OF LITAF AND MIFS GENES AFTER PAMPS STIMULATION

(A) MIFs genes: Treatments with bacterial LPS and LTA did not induce any significant variation. Only zymosan and poly I:C treatments induced a significant increase of expression after 24 h.

(B) LITAF gene: LPS, zymosan and Poly I:C induced LITAF expression. LTA was not able to induce it. Those results could suggest that **only recognition of foreign particles by specific TLRs are able to** initiate a signal transduction cascade leading TNF-a production through **LITAF activation.**

CONCLUSIONS:

Amoeboid-phagocytes are the most active immune cells involved in phagocytosis and ROS production. Moreover, the phagocytic response can be enhanced by cooperation between phagocytes and spherulocytes and is also modulated by PAMP stimulation. Coelomocytes are also activated by bacterial components as it is suggested by the high expression levels of MIF and LITAF genes .