TRIPLE LUX-B: PHAGOCYTOSIS IN MUSSEL HEMOCYTES

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ABSTRACT

The TRIPLELUX-B Experiment contributes to risk assessment concerning immunotoxicity under space flight conditions. The assay system of the TRIPLELUX-B Experiment will be performed with a well defined quantification and evaluation of the immune function phagocytosis. The indicator cells are the hemocytes of blue mussels (Mytilus edulis) and oysters (Crassostrea gigas). The signals of the immuno cellular responses are translated into luminescence as a rapid optical reporter system. The results expected will allow to conclude wether the observed responses are caused by microgravity and/or radiation. The immune system of invertebrates has not been studied so far in space.
The choice of the phagocytes from invertebrates is justified by the claim to study the universal validity of innate immune responses. The components of the phagocytosis test system for the BIOLAB are now established under terrestrial conditions. The next step for adaptation to the BIOLAB conditions and hardware is in progress.

1. INTRODUCTION

The definition of the immune response by the mussel hemocytes is the selective reaction to particles which are identified as foreign by its immune system shown by phagocytosis. The phagocytotic activity is based on the chemotaxis (migration assay) and adhesion, ingestion and phagosome formation. The attachment at the surface of the hemocytes [1] and consequently the uptake of the particles or bacteria can be quantified in the format of a luminescent assays [2] or a fluorescent assay [3, 4]. Another relevant endpoint of the phagocytosis is the oxidative burst measured by luminescence.

2. OBJECTIVES

Measuring phagocytosis of hemocytes in primary culture (suspended cells) derived from oysters or blue mussels. The model: invertebrate immune cells.

3. MATERIALS AND METHODS

The hemolymph and the hemocytes are obtained from the posterior adductor muscle sinus of the individual mussels using a one way syringe. Beside the direct uptake and phagocytosis [2,3,4] of particles the production of reactive oxygen species can be quantitatively measured as oxidative bursts with luminol [5, 6, 7].
An important criteria to maintain the viability of the cells in culture is to have a nutrition media that serves on the one hand the needs of the cells [8] and their immuno response.

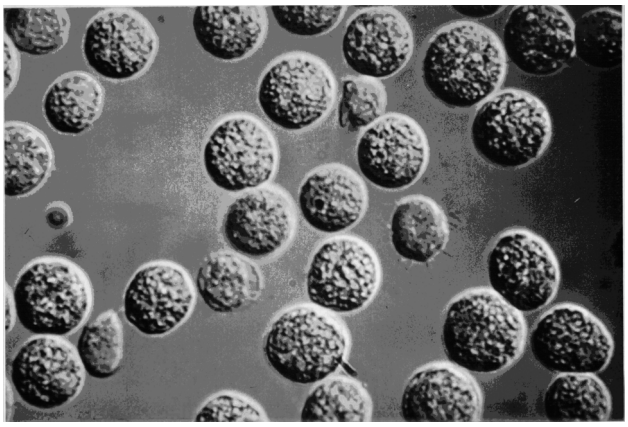

Fig. 1. Hemocytes (granulucytes) of *Mytilus edulis*

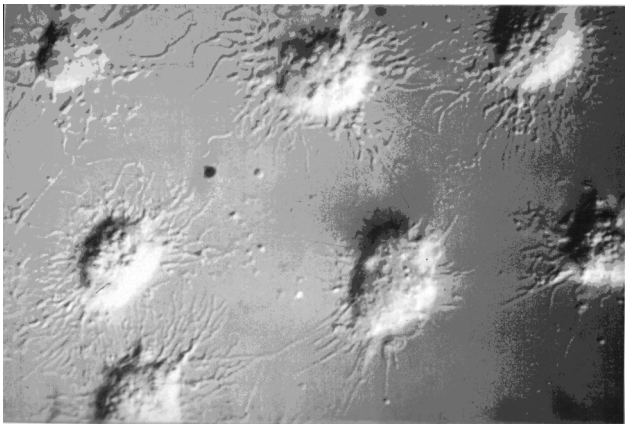

Fig. 2. Hemocytes with stretching Pseudopodia ready for phagocytosis

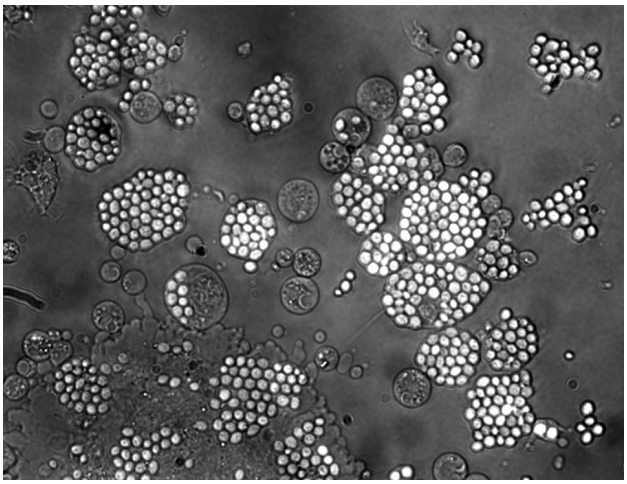

Fig. 3. Phagocytosis and luminescence-labelled bacteria

4. RESULTS AND DISCUSSION

The hemocytes are easy to obtain and to keep under standardised conditions. The shelve life is expanded to 11 month. There are many reference data from laboratory and field investigations concerning the measurement of direct phagocytosis by mussel hemocytes [3,4,5,6,7,.9,10] The results of the oxidative burst measurements are done under laboratory conditions, the calibration under BIOLAB criteria is in progress.

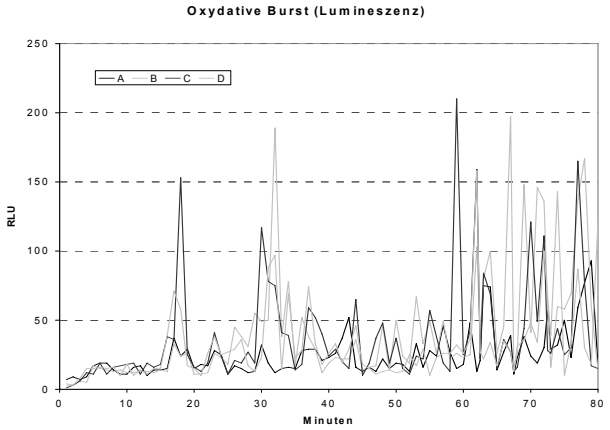

Fig. 4. Oxidativ burst events induced by Zymosan and measured as luminol luminescence

The optimisation of the testsystem is in progress by investigations concerning the storage of froozen cells and revitalisation in the context of phagocytosis.
Further investigations are focussed on the optimisation of testing the cell viability under flight conditions, the testing of biocompatibility of the in-flight equipment as well as the optimisation of signal detection and data processing by the in-flight signal detection equipment.

5. REFERENCES

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