P-024 **Secretomic profile of cystic fibrosis cells encapsulated in alginate microbeads**

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INTRODUCTION

Cystic fibrosis (CF) is an autosomal recessive disorder caused by mutations of the CF transmembrane conductance regulator (CFTR) gene, which encodes a transmembrane protein present on a variety of cell types and organelles (Welsh 1995). Chronic bacterial infection of the airways, thick airway mucous, and bronchiectasis, characterize the CF lung. The excess mucus is largely caused by the influx of neutrophils, attracted to the site by the increased expression of chemokines such as interleukin-6 (IL-6) and interleukin-8 (IL-8) by bacterial products and inflammatory cytokines. In order to study in detail the mechanism(s) of activation of IL-8 in CF, the IB3-1 cell system has been recently proposed. IB3-1 is a bronchial epithelial cell line, derived from a CF patient with a CFTR genotype of F508del/W1282X, therefore carrying the associated cystic fibrosis mutation (Bezzeri 2008). This cell line can be induced to high expression of pro-inflammatory proteins, following infection with Pseudomonas aeruginosa or by treatment with TNF-α. In both cases, 24 hours treatment with TNF-α is usually sufficient to induce, in IB3-1 cells, a deep alteration of mRNA expression and protein secretion profile with a typical increase of IL-6 and IL-8 mRNA and IL-6/IL-8 release. Thus, it would be of great interest to develop a specific system that would assist elucidate the mechanism of bacterial activation of IB3-1 cell, as well as the effect of the secreted chemokines on target cell populations, in co-culture experiments. For instance, IB3-1 cells could be co-cultured with Pseudomonas aeruginosa or polymorphonuclear cells (PMN), the major phagocytic cell of blood and also with other inflammatory cells such as basophils, eosinophils and T-cells (Dimango 1995). The co-culture experiments could be performed in the presence/absence of a semipermeable membrane embedding the IB3-1 cells, representing a physical barrier to cell/cells interactions but allowing the cross-talking among the different cells mediated by soluble factors. In this respect, polysaccharidic based microbeads represent one of the most intensively studied system to immunoisolate cells or cell clusters, because of the spherical shape and the small size that offers an optimal surface to volume ratio and an optimal diffusion capacity (De Vos 2006).

The technology of cell microencapsulation is based on the immobilization of living cells within a polymer matrix, often alginate, that constitutes a semipermeable membrane. These "living" delivery systems have been proposed for controlled and continuous expression of a number of compounds including hormones, growth factors and biological response modifiers.

In the present study we, report the encapsulation of IB3-1 cells in polymeric microbeads as well as their secretomic profile when cultured in standard conditions or after stimulation with TNF-α.

MATERIALS AND METHODS

IB3-1 cells were obtained from LGC Promochem. Monodisperse alginate beads containing IB3-1 cells were prepared using an air-driven droplet generator for cell encapsulation. Before encapsulation, confluent monolayers of IB3-1 were scraped off by 0.05% trypsin/EDTA (Gibco, Grandisland, NY, USA) (2 min), washed with PBS, counted by hemocytometric analysis and assayed for viability by double staining with propidium iodide (PI) and Calcein-AM (Sigma). IB3-1 cells were suspended in a 1.5% (w/v) aqueous solution of highly purified sodium alginate (Inotech, Dottikon, Switzerland). The resulting cell suspension was continuously aspirated by a syringe pump and extruded through the air-driven droplet generator, under sterile conditions. The generated microdroplets were harderned by an ionotropic gelling process into a 1.2 % (w/v) barium chloride solution that resulted in the production of barium alginate microbeads. The morphology of barium alginate microbeads was evaluated by optical microscopy and stereomicroscopy (Nikon microscopes, Tokyo, Japan). After encapsulation and after different lengths of time, the viability of IB3-1 cells was analyzed by double staining with propidium iodide (PI) and Calcein-AM, following manufacturer's instructions. Equal quantity ($20x10^6$ cells) of free and encapsulated cells (derived from the same flask) were treated with 80 ng/ml TNF-α for 24 hours and cytokines in tissue culture supernatants were measured by Bio-Plex cytokine assay (Bio-Rad Laboratories, Hercules, CA) as described by the manufacturer. 50 μl of cytokine standards or samples (supernatants recovered from treated cells) were incubated with 50 μl of anti-cytokine conjugated beads in 96-well filter plates for 30 min at room temperature with shaking. Plates were then washed by vacuum filtration three times with 100 μl of Bio-Plex wash buffer, 25 μl of diluted detection antibody were added, and plates were incubated for 30 min at room temperature with shaking. After three filter washes, 50 μl of streptavidin-phycoerythrin was added, and the plates were incubated for 10 min at room temperature with

shaking. Finally, plates were washed by vacuum filtration three times, beads were suspended in Bio-Plex assay buffer, and samples were analyzed on a Bio-Rad 96-well plate reader using the Bio-Plex Suspension Array System and Bio-Plex Manager software (Bio-Rad Lab, Hercules, CA).

RESULTS AND DISCUSSION

IB3-1 cells were embedded into alginate microbeads using an air-driven droplet generator for cell encapsulation. The encapsulation procedure was relatively simple and consisted of a limited number of steps. In order to mimic physiological conditions that are essential for mammalian cells, the entire procedure was conducted at room temperature, under physiologic pH and tonicity using a pyrogen-free alginate. The hardening of the generated alginate microdroplets was accomplished by an ionic gelation procedure, using barium chloride solution. The resulting barium alginate microbeads were elastic and transparent, thus facilitating the microscopic observation of cell morphology and viability, during the in vitro studies. The IB3-1 cells were assayed by "live/dead cells" test, before and after encapsulation in all cases the determinated viability was ≥ 90%, as indicated by the fluorescence photomicrograph reported in the insets of Figure 1 A and B.

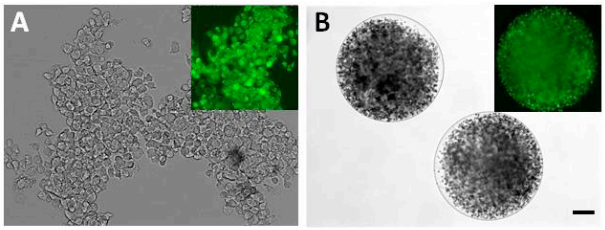

Figure 1: optical (A, B) and fluorescence (insets) microphotographs of alginate microparticles containing IB3-1 cells. The bar corresponds to 60 (A) and 150 μm (B).

Free and encapsulated IB3-1 cystic fibrosis cells were treated for 24 hours in the presence of 80 ng/ml of TNF-α. After this treatment, microcapsules were separated from the culture medium, which was analyzed for content of IL-6 and IL-8 by Bioplex analysis. The Bio–Plex 200 multiplex suspension array represents, in fact, an assay system, employing Luminex xMAP–technology, for the simultaneous detection and quantitation of multiple bioanalytes (proteins, peptides, DNA and RNA) in a single microplate, requiring only very small sample volumes.

Minor differences in the amounts of secreted IL-6 and IL-8 protein levels are present when mediums isolated from cultures of free and encapsulated IB3-1 cells are compared (data not shown). On the contrary, release by TNF-α treated cells is compared to that of control IB3-1 cells, the levels of IL-6 and IL-8 increase significantly both in free and in encapsulated IB3-1 cells (Fig. 2). The TNF-α mediated fold induction of IL-8 release was found to be similar in free and encapsulated IB3-1 cells, while in the case of IL-6, the fold increase was more evident in free IB3-1 cells.

CONCLUSIONS

In summary, the paper describes(a) the encapsulation for IB3-1 cells in alginate microbeads following a microencapsulation procedure developed in our laboratory that is based on the generation of monodisperse droplets by an air-driven droplet generator for cell encapsulation; (b) the characterization of alginate microbeads produced; (c) the determination of viability of the encapsulated IB3-1 cells and finally (d) the characterization of the encapsulated IB3-1 cells, in term of secretomic profile, analysing the culture medium by Bio-Plex strategy. The significant finding of the study was the evidence of the induction of IL-6 and IL-8 from encapsulated IB3-1 cells.

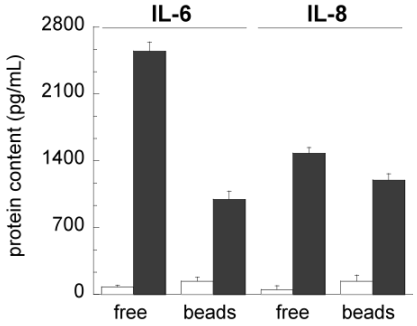

Figure 2: release of interleukins 6 and 8 (IL-6, IL-8) by IB3-1 cells. Both free and encapsulated cells were cultured for 24 hours, in the absence (open bars) or in the presence of TNF-α (80 ng/ml) (closed bars). Data represent the average of three independent experiments±SD.

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