



## BIOLOGICAL ACTIVITY OF HETEROLOGOUS MURINE INTERLEUKIN-10 AND PRELIMINARY STUDIES ON THE USE OF A DEXTRIN NANO GEL AS A DELIVERY SYSTEM

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### KEYWORDS

Protein delivery system; anti-inflammatory cytokine; interleukin-10; endotoxin-activated macrophages; dextrin nanogel.

### INTRODUCTION

Interleukin-10 (IL-10) is an anti-inflammatory cytokine, which active form is a non-covalent homodimer with two intramolecular disulphide bonds that are essential to its biological activity, which includes reduction of tumor necrosis factor  $\alpha$  (TNF- $\alpha$ ) synthesis and down-regulation of class II major histocompatibility complex (MHC-II) molecules on monocytes/macrophages (de Waal Malefyt, 1991). Due to IL-10 potential applications in various medical fields, it is essential to develop systems that can effectively deliver the protein. A promising system is protein encapsulation by polymeric nanoparticles (NPs), which minimize denaturation, and enables slow-release, while maintaining an effective concentration for the necessary period of time. In previous work, we have developed and characterized NPs obtained by self-assembling of hydrophobized dextrin (Gonçalves, 2007) whose properties makes them promising for IL-10 delivery.

### METHODS

The recombinant mutated (C149Y) murine IL-10 (rIL-10) was expressed, in form of inclusion bodies, in *E. coli* BL21 star. rIL-10 was recovered by a process of solubilization in 6M guanidine, renaturation and re-oxidation of disulphides. The dimeric form of rIL-10 was collected by gel filtration and further purified by ion-exchange chromatography. SDS-PAGE analysis was used to confirm molecular weight and purity. The amount of rIL-10 obtained was quantified by ELISA. rIL-10 bioactivity was assayed by its ability to inhibit the production of TNF- $\alpha$ , quantified by ELISA, and the surface expression of MHC-II molecules, monitored by FACS analysis, in lipopolysaccharide (LPS) and IFN- $\gamma$  activated bone marrow derived macrophages (BMDM).

To form the self-assembled NPs, lyophilized dextrin-VMA-SC<sub>16</sub> was resuspended in culture medium, at room temperature. NPs formation was confirmed by dynamic light scattering. The complex NPs/rIL-10 was formed by dissolving rIL-10 in culture medium and then by mixing lyophilized dextrin-VMA-SC<sub>16</sub>. rIL-10 incorporation into NPs was verified quantifying the amount of rIL-10 free in solution by ELISA. The IL-10 stability was accessed by circular dichroism (CD). The

release of rIL-10 from the complex NPs/rIL-10 was assessed in a BMDM culture, by measuring free rIL-10 by ELISA. Bioactivity of rIL-10 released from the NPs/rIL-10 complex was assayed as described previously.

### RESULTS

SDS-PAGE confirmed the expression and molecular weight (18 kDa) of the rIL-10 as well as their purity. Total recovery of dimeric rIL-10 was about 1-1.5 mg/L culture, quantified by ELISA.

rIL-10 (in the range of 0.1 to 250 ng/mL) shown the ability to inhibit the TNF- $\alpha$  production and to reduce MHC-II expression on stimulated BMDM (figure 1A and B).

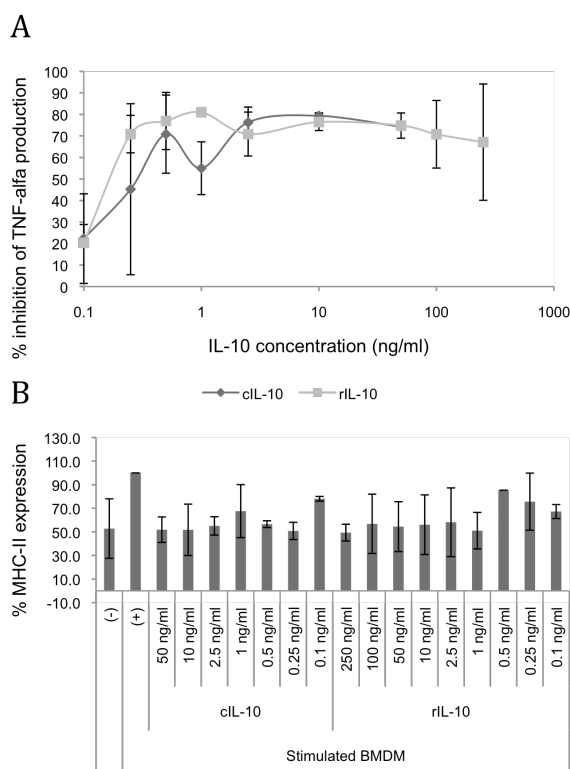


Figure 1: Biological activity of rIL-10 and cIL-10. (A) Percentage of inhibition of TNF- $\alpha$  production, by 0.1 ng/ml LPS and 1.0 ng/ml INF- $\gamma$  stimulated BMDM. (B) Percentage of MHC-II expression induced by cIL-10 and rIL-10 treatment of stimulated BMDM. Data points are the means  $\pm$  SD. (+) – positive control of macrophage activation; (-) – negative control of macrophage activation.

rIL-10 incorporation by NPs was confirmed by quantifying, the amount of rIL-10 in free in solution,

that was negligible. The secondary structure of free soluble rIL-10 is mainly helical (Zdanov, 1995), and the complexation in the nanogel did not induce any conformational change (figure 2). Further CD studies showed that rIL-10 stability is significantly increased when it is complexed with the dextrin nanogel.

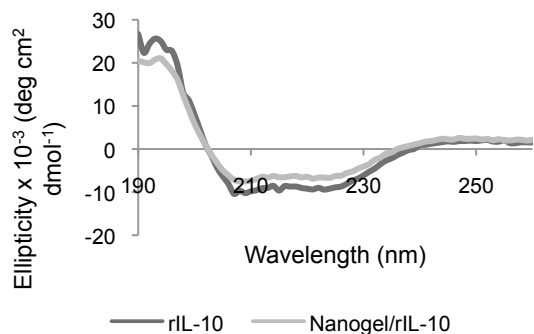
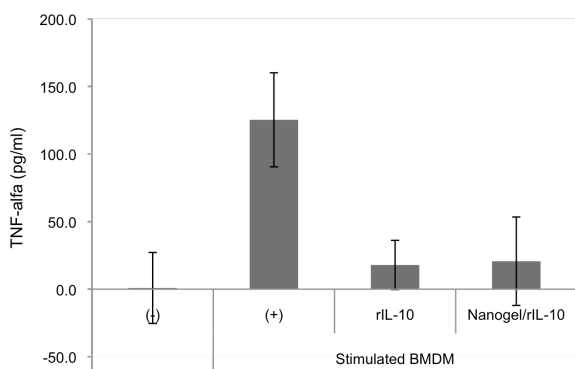


Figure 2: CD spectra of free rIL-10 (0.25 mg/ml) and complex nanogel/rIL-10 (1 mg/ml nanogel and 0.25 mg/ml rIL-10) at 37°C in PBS.

In the presence of 20% serum, rIL-10 is being released over time in a BMDM culture. After two hours of incubation with the NPs/rIL-10 complex, the rIL-10 reaches a maximum concentration; a stable value of about 35 ng/mL rIL-10 being estimated after twenty-four hours.

A



B

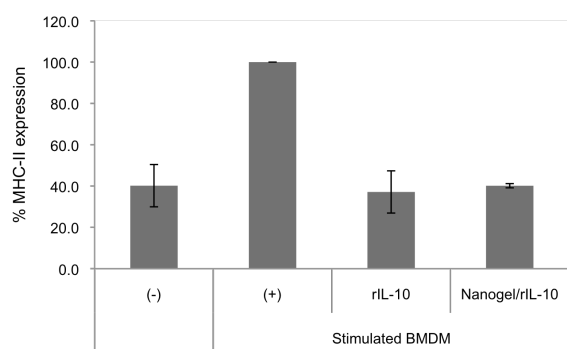


Figure 3: Biological activity of rIL-10 released from nanogel/rIL-10 complex. (A) TNF- $\alpha$  concentration (pg/ml), produced by 0.1 ng/ml LPS and 1.0 ng/ml INF- $\gamma$  stimulated BMDM, treated with rIL-10 and nanogel/rIL-10. (B) MHC-II induced, in stimulated BMDM, by 50 ng/ml rIL-10 and nanogel/rIL-10. Data points are the means  $\pm$  SD. (+) – positive control of

macrophage activation; (-) – negative control of macrophage activation.

The rIL-10 released from the NPs/rIL-10 complex was able to inhibit TNF- $\alpha$  production and MHC-II expression at the same level as the soluble rIL-10 (figure 3A and B).

## DISCUSSION & CONCLUSIONS

A mutated form of murine IL-10 was successfully expressed in *E. coli*. This recombinant protein was recovered and purified from inclusion bodies and demonstrated biological activity similar to a commercially available IL-10. Dextrin self-assembled nanogel was able to efficiently encapsulate and protect rIL-10 from denaturation at 37°C, and also enables rIL-10 to be released in biologically significant amounts over time. The biological activity of the released rIL-10 was confirmed by the evaluation of the production of TNF- $\alpha$  and expression of MHC-II on endotoxin-stimulated BMDM. The simplicity of the preparation of the nanogel/rIL-10 complex, associated to the enhancement of protein stability and controlled release, makes this a very promising system.

IL-10 has potential application in various medical fields, such as in acute inflammatory diseases, namely rheumatoid arthritis, and these results point to dextrin nanogel as a promising carrier of IL-10, which enables the protein sustained release.

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**VERA CARVALHO** was born in V. N. Famalicão, Portugal. In 2002 got her degree in Biochemistry in Universidade do Porto. In 2006 concluded her master degree in Biomedical Engineering also in Universidade do Porto. Since 2002 she had several grants in the Biological Engineering Department of Universidade do Minho where she researches in the biomedical and biotechnology areas.

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