



## The Role of Polyamines in Activation of Macrophages .

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### Abstract

The polyamines are small molecules cationic that occurs naturally in eukaryotic cells. Part of polyamines are the putrescine, spermidine and spermine with roles in cell development, proliferation and cell differentiation. The macrophages are extremely versatile and plastic cell from innate immunity, which are responding the environment influences. Macrophages can be classified as pro-inflammatory (M1) and anti-inflammatory (M2). The first profile yield cytokines as TNF- $\alpha$  and IL-1 $\beta$ , with properties anti-tumoral response, anti-microbial and the glycolytic metabolism. The second profile yield cytokines as IL-10 (M2), with proprieties wound healing and the oxidative phosphorylation metabolism. Macrophages treated with polyamines could change from an pro-inflammatory to anti-inflammatory profile or reduce the pro-inflammatory profile.

### Key words:

Macrophages, polyamines, polarization

### Introduction

The innate immunity is the first defense against microorganisms, and also acts in maintenance of homeostasis and tissue repair. It is able to responde for cell damage and molecular patterns associated with pathogens in the rapid and efficient way<sup>1</sup>. The macrophages are antigen-presenting cell (APC) with different function and acting in scenario of inflammation, phagocytose, immunoregulation and repair tissue. Macrophages can be presented the profiles pro or anti-inflammatory.

Researchs in polyamines pathways and mecanism, could change or reduce pro-inflammatory profile, stablishing in the future a new therapeutics mecanism to control inflammatory diseases. Despite the polyamines in literature show therapeutics proprieters, details of polyamines mecanisms and how is working still unclear and need be investigated. Apparently, the polyamines have a role in macrophage polarization. For trigger the polyamine cycle is necessary the aminoacid arginine metabolism in the pro or anti-inflammatory way. In the pro-inflammatory way, the argine is converted in the anti-microbial Nitric Oxide (NO) by Synthase Nitric Oxide (NOS). In the anti-inflammatory way, the arginine is converted by arginase-1 in ornithine that is converted by ornithine descarboxilase (ODC) in putrescine, the first step of polyamines<sup>2</sup>. Like a downstream reaction, the others spermidine and spermine polyamines rely on the first step to be builded. So, we believe the polyamines have a important role in polarization of macrophages. Through this, our goal is understand the immunologic effect of polyamines in each macrophage profile.

### Results and Discussion

The role in polyamines in health and its benefits are cited in literature, but information about doses of polyamines and how the polyamines mecanims acts still unclear. Our first aim was to test the viability of BMDMs treated with different doses of putrescine, spermidine and spermine (5  $\mu$ M to 1000  $\mu$ M) for 30 minutes and activated for 6 hours with LPS (100 ng/mL). After this process, we collect the supernatant of each plate treated with each polyamines, and was applyied Trypan Blue to see whether the macrophages were died when treated with this doses.

They were counted in Neubauer camara. Values above 500  $\mu$ M polyamines doses induced a higher number of macrophages death. The viability tests shown that better polyamines doses to use in BMDM were to putrescine around 100  $\mu$ M, spermidine 50  $\mu$ M, spermine 20  $\mu$ M.

We analyze by ELISA procedure, TNF- $\alpha$ , IL-6 and IL-10 cytokines from BMDM supernatant.

Increasing doses of putrescine 0.1 until 4 mM blunt TNF- $\alpha$ , but the spermidine and spermidine did not show alteration. Increasing doses of putrescine and spermidine between 5 until 100  $\mu$ M, did not show alteration in concentration of IL-6. For spermine doses of 20  $\mu$ M did not shown alteration, but higher doses 100 and 250  $\mu$ M have shown to reduce IL-6 release.

In spermine doses of 20  $\mu$ M have shown reduction of IL-10. Others polyamines need to be investigated with IL-10 secretion.

Higher doses of polyamines presented negative effects about macrophages resulting in cell death. Polyamines compounds shown differences influences in cytokines secretion of TNF- $\alpha$ , IL-6, IL-10. Putrescine has been more positive effective in reduction TNF- $\alpha$  and spermine, despite higher doses, is reducing IL-6.

### Conclusions

Apparently, putrescine has more positive effective in reduction TNF- $\alpha$  and spermine, despite higher doses, in reducing IL-6. More experiments are required to fully undertand the mechanism of polyamines.

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<sup>1</sup>BISWAS, S. K. et al. Macrophage polarization and plasticity in health and disease. *Immunol Res*, v. 53, n. 1-3, p. 11-24, Sep 2012. ISSN 0257-277x. Disponível em: < <http://dx.doi.org/10.1007/s12026-012-8291-9> >.

<sup>2</sup>MADEO, F. et al. Spermidine in health and disease. *Science*, v. 359, n. 6374, Jan 26 2018. ISSN 0036-8075. Disponível em: < <http://dx.doi.org/10.1126/science.aan2788> >.