Effect of garlic, in the absence or presence of lipopolysaccharide, on tumor necrosis factor alpha secretion from macrophages plated on different surface areas

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Abstract

It has been known for many years that garlic is good for your immune system. Macrophages are very important cells of the innate immune system that respond to pathogens. One of the ways macrophages respond to pathogens is by producing cytokines. Tumor necrosis factor alpha (TNFα) is an important cytokine of innate immunity. Lipopolysaccharide (LPS) is a bacterial cell membrane component that stimulates TNFα secretion from macrophages. In our lab, we have found that garlic stimulates TNFα secretion from the murine macrophages cell line J774A.1 cells in the absence and presence of LPS. The objective of this project was to determine whether the effect of garlic on cell TNF-α production was dependent on plate surface area. Thus, we plated cells at 1.25x10⁵ cells/ml in 96-well, 48-well, 12-well or 6-well plates. For the 96-well plates, 200μl of cells were plated, 48-well plates, 1ml of cells were plated, 12-well plates, 2ml of cells were plated, and two 6-well plates, 5ml of cells were plated. Twenty four hours later, the cells were treated with garlic (G) diluted 1:500 in pyrogen-free water (PFW) in the absence or presence of LPS (0.1mg/ml). Twenty four hours later, cell supernatants (195ul) were collected from each well, independent of plate format, and stored -70°C until analysis. TNF-α levels were then determined from cell supernatants by cytokine specific enzyme-linked immunosorbent assay (ELISA). We found that garlic stimulates TNF-α in the absence or presence of LPS regardless of the plate format. We believe that garlic enhances the immune function thereby helping macrophages better respond to pathogens.

Materials and Methods

Garlic (G) is extracted with water

J774A.1 macrophages were plated at 1.25X10⁵/well in 96-well plate, 48-well plate, 12-well plate and 6-well plate for 24 hours.

24h after plating, cells were treated with LPS (0.1μg/ml), G1:500 dilution, and LPS+G1:500 and incubated for 24 hour.

Cell supernatant was collected 24h after treatment and stored at -70°C until analyzing TNF-α levels

TNF-α is determine by ELISA

Garlic stimulates TNF-a secretion similarly from J774A.1 macrophages in 96-well and 48 well plates

Garlic stimulates TNF-a secretion similarly from J774A.1 macrophages in 96-well and 6 well plates

Garlic stimulates TNF-a secretion similarly from J774A.1 macrophages in 96-well and 12 well plates

Garlic stimulates TNF-a secretion from J774A.1 macrophages in the absence and presence of LPS in all plate formats

Summary

• Garlic stimulates TNF-α from J774A.1 macrophage.
• LPS stimulates TNF-α to a greater extent than Garlic.
• Garlic slightly stimulate LPS induced TNF-α.
• Same results were obtain when using 96 well, 48 well, 12 well, and 6 well plate.

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