Content of Research Report


B. Abstract:

Garlic (*Allium sativum*) has antiparasitic, antibacterial, and antifungal properties, specifically against the yeast *Candida albicans* (*C. albicans*). Garlic is also known to boost the immune system. The immune system consists of innate and adaptive immunity. Macrophages, phagocytic cells that engulf pathogens and dead cells, play a big part in the innate immunity and are major producers of an important pro-inflammatory cytokine, tumor necrosis factor-alpha (TNF-α), which is released upon the detection of antigens such as *C. albicans*. In our lab we have observed that garlic suppresses *C. albicans*-induced TNF-α from J774A.1 murine macrophages. We first tested to see whether cell density altered the effect of garlic. Thus, macrophages were seeded at four densities: 0.625x10^5 cells/mL, 1.25x10^5 cell/mL, 2.50x10^5 cells/mL, 5.00x10^5 cells/mL. We found that garlic decreases *C. albicans*-induced TNF-α secretion at all cell densities.

Next, we wanted to investigate the signaling mechanisms by which garlic suppresses *C. albicans* induced TNF-α, specifically the involvement of the mitogen-activated protein kinases (MAPKs). MAPK activation induces TNF-α secretion from macrophages. We first examined the involvement of ERK1/2 in *C. albicans*-induced TNF-α secretion by treating with pyrogen free water (PFW, the garlic diluent), garlic, and heat killed *C. albicans* with or without garlic in the presence and absence of the ERK1/2 inhibitor. Our preliminary findings suggest that ERK1/2 may not be involved in garlic’s effect on *C. albicans*-induced TNF-α secretion. Investigating garlic’s effect and its mechanism on *C. albicans* induced TNF-α production will help develop a better understanding of garlic as an antifungal agent, particularly against a *C. albicans* infection, within our innate immune system.

C. Major Objective(s):

1) To see if the different macrophage cell densities change the effect of garlic.

2) To investigate garlic's mechanism via the MAPK pathways in suppressing the *C. albicans* induced TNF-α secretion from macrophages.
D. Background research with analysis and summary of literature review:

*Allium sativum* (garlic) has been used for medical purposes for thousands of years in many different cultures. A component in freshly crushed garlic, allicin, is known for its antibacterial, antiparasitic, antiviral and antifungal properties, including activities against the yeast *Candida albicans* (*C. albicans*) (Ankri and Mirelman, 1999). Today, some of garlic's uses include helping to prevent atherosclerosis, high blood pressure, high cholesterol, and boosting the immune system (Ehrlich, 2011).

The immune system is composed of an innate and an adaptive immunity. Within the innate immune system, important cells, known as macrophages, phagocytize pathogens and dead cell debris (Mosser and Edwards, 2008). In addition, they release cytokines, proteins that participate in immune cell communication. Macrophages are main producers of tumor necrosis factor-α (TNF-α), an important pro-inflammatory cytokine (Parameswaran and Patial, 2010). TNF-α is involved in a variety of cell functions including cell proliferation, differentiation, and apoptosis (www.ncbi.nlm.nih.gov/gene/7124, 2013). Irregular production of TNF-α have been linked to stimulation of various inflammatory diseases such as rheumatoid arthritis, Crohn’s disease, and atherosclerosis (Parameswaran and Patial, 2010).

In our laboratory, we have found that garlic reduces *C. albicans*-induced TNF-α secretion from J774A.1 murine macrophages when these cells were seeded at 0.625x10^5 cells/mL, 1.25x10^5 cell/mL, 2.50x10^5 cells/mL, 5.00x10^5 cells/mL. Presently, we are investigating the mechanism via signaling pathways in which garlic decreases the *C. albicans* induced-TNF-α secretion. Our preliminary findings suggest that the mitogen activated protein kinase pathway may not be involved in garlic’s effect on reducing *C. albicans* induced-TNF-α.

E. Methods (Experimental procedure/design):

An aqueous garlic extract was prepared in pyrogen free water (PFW). The *C. albicans* strain CP680 was suspended in Sabouraud dextrose Bouillon (SAB) and incubated at 25°C. After 24h, the yeast was heat killed in 3mL aliquots in an Erlenmeyer flask over a Bunsen burner flame for 35 seconds, cooled on ice, and flamed for another 20 seconds to ensure death. J744A.1 murine macrophages were seeded in a 96-well plate at four densities: 0.625x10^5 cells/mL, 1.25x10^5 cell/mL, 2.50x10^5 cells/mL, 5.0x10^5 cells/mL. The
macrophages were then incubated at 37°C in 5% CO₂ for 24h. The cells were treated with PFW, garlic extract, and heat killed C. albicans in the presence and absence of garlic. After another 24h of incubation, the cell supernatants were collected and stored at -80°C until the amount of TNF-α was determined by enzyme-linked immunosorbent assay (ELISA).

To examine the signaling mechanism in which garlic suppresses C. albicans induced TNF-α secretion, the macrophages were seeded in a 96-well plate at 1.25x10⁵ cell/mL. After 24h incubation at 37°C in 5% CO₂, the cells undergoing experimental treatment were treated with DMSO and PD, the inhibitor or the ERK1/2 pathway; they were then incubated for 1h. After the 1h, the cells were treated with PFW, garlic extract, and heat killed C. albicans; the inhibitor PD and DMSO were then added back into the wells. The cells were incubated for 8h; the cell supernatant was collected and stored at -80°C until the ELISA assay.

F. Results:

In the presence of garlic, there is a slight stimulation of TNF-α secretion at all four cell densities compared to the control (PFW) treatment. When challenged with C. albicans, there was a dramatic increase in the TNF-α level. However, when the cells were treated with the yeast and the garlic extract, there is a significant decrease in C. albicans induced TNF-α secretion across all the cell densities.

We see the same effect of garlic in the presence of the inhibitor PD: there is a significant decrease in the C. albicans induced TNF-α. However, in the presence of PD the level of TNF-α induced by C. albicans, with or without garlic, is remarkably lower compared to the controls.

G. Discussion:

It seems that garlic is able to suppress C. albicans induced TNF-α regardless of the macrophage cell densities. Also, so far, our investigation on the signaling mechanism in which garlic suppresses C. albicans induced TNF-α secretion suggests that garlic may not or may not exclusively work through the ERK 1/2 pathway. I will also employ the inhibitors SB and SP for the p38 and JNK pathways respectively and observe the effect of garlic on the C. albicans induced TNF-α secretion.
In the presence of the inhibitor PD, there is a notable decrease in the *C. albicans* induced TNF-α secretion with or without garlic compared to the control treatments. This may be due to the cell toxicity effect of the inhibitor PD. I will continue to carry out cell viability assays including the XTT assay and possibly along with cell counting, then compare the results to examine the effect of PD on the cells' viability.

In summary, our findings suggest that garlic reduces the inflammatory response induced by the *C. albicans*. This finding is in agreement with those of others who have found that garlic has anti-inflammatory properties (Lang et al., 2004).
H. References (Bibliography)


<http://umm.edu/health/medical/altmed/herb/garlic>


