In Vitro Rate of Phagocytosis in Macrophages Stimulated by

*Astragalus membranaceus*

Current research suggests that *A. membranaceus* increases the efficiency of the immune system. IC-21 murine macrophages were exposed to a 1% tincture of *A. membranaceus*. After stimulation for 24 hours, a phagocytosis assay was performed and it was determined that *A. membranaceus* does have a positive effect on the rate of phagocytosis in these cells.

**Introduction**

The use of herbal supplements to stimulate the immune system has been used by eastern medicine for thousands of years. Current research has shown that these herbal supplements increase the effectiveness of leukocytes, thus increasing the effectiveness of the immune system (1). *Astragalus membranaceus* is one such supplement that is commonly taken at the first signs of an infection also known as general malaise. In recent years the amount of research done with *A. membranaceus* has increased significantly. One such study showed that *A. membranaceus* increases the production of interleukins in mice *in vivo* (2). It has also been shown that *A. membranaceus* increases the amount of antibody production in mice *in vivo* (3). The increased production of interleukins and antibodies may be attributed to many factors, including an increase in phagocytosis.

The macrophage is an important leukocyte in the immune system, specifically in its ability to phagocytose (4). It is also an effective antigen presenting cell making it important in activating the acquired immune response. With an increased rate of phagocytosis a speedier and more effective immune response would soon follow. When IC-21 macrophages are subjected to *A. membranaceus* the
rate of phagocytosis is expected to increase. To fully understand *A. membranaceus* and its effects on the rate of phagocytosis in IC-21 macrophages a series of phagocytosis assays were performed.

**Methods**

**Cell Culturing**

IC-21 (ATCC TIB-186) mouse macrophages were cultured in RPMI-1620 media supplemented with 10% fetal bovine serum and 1% pen/strep. Macrophages were incubated at 37° in 5% CO₂ with a relative humidity of 100%.

**Media Preparations**

Two separate media were created; media containing 1% *A. membranaceus* tincture (70% *A. membranaceus* root and 30% ethanol) and media containing 0.3% ethanol.

**Phagocytosis Assay**

Fully confluent IC-21 mouse macrophages were lifted with Ca²⁺ free PBS and 3.0x10⁵ cells were transferred to each micro-chamber well (Lab-Tek chamber slide, 8 micro-chambers). An equal volume of RPMI media was added to each well and macrophages were allowed to reattach for three hours. 1% *A. membranaceus* supplemented media, RPMI media alone and 0.3% ethanol control media were added to respective wells. Cells were allowed to metabolize supplements for 24 hours.
The media was removed and latex beads (Sigma L1030) were added to macrophages at a concentration of 20 beads per cell. Respective media were replaced and macrophages were allowed to phagocytose at 37° for a period of 60 or 90 minutes. Micro-chambers were washed three times with respective media to remove loose beads.

**Slide Staining**

The macrophages were stained with Protocol Hema 3 stain (Fisher series) and viewed using a Leica DMIRB inverted scope (40x magnification). A series of eight randomized bright field pictures were taken in each well. 580 nm ultraviolet light pictures were taken and superimposed over bright field picture to amplify bead luminosity.

**Statistically Analysis**

To determine the rate of phagocytosis this equation was used: (cells that have phagocytosed)/ (total number of cells per field of view) and (cells that have phagocytosed more than one bead)/ (total number of cells per field of view). Averages and standard deviations were calculated and a two sample single tail t test (CI=95%) was performed using Excel statistical software.

**Results**

**Phagocytosis**

We wanted to explore if there would be an increase in the rate of phagocytosis when macrophages were exposed to *A. membranaceus*. The cells were
exposed to a 1% concentration of *A. membranaceus* and allowed to phagocytose for a period of 60 or 90 minutes with nine replicates. The rate of phagocytosis in both the ethanol control and the media alone were identical, p=0.39. The average rate of phagocytosis of macrophages subjected to *A. membranaceus* for 60 minutes was 1.64 % (±0.60%), p=0.00054 and 1.61% (±0.82%), p=0.00046 for 90 minutes (fig 1). We determined that the rate of phagocytosis in macrophages increased when subjected to 1% *A. membranaceus* and allowed to phagocytose for both 60 and 90 minutes.

**Phagocytosis of Multiple Beads**

We expected to see an increase in the amount of beads each macrophage phagocytosis, since this would also indicate an increased rate of phagocytosis. The macrophages were tested similarly to the prior experiment; however macrophages that phagocytosed more than bead were only recorded. The rate of phagocytosis of more than one bead for both the control, ethanol control and 1% *A. membranaceus* for 60 minutes were close to the same, p=0.38 and 0.44. The average rate of phagocytosis in macrophages subjected to *A. membranaceus* and allowed to phagocytose for 90 minutes was 0.27% (±0.26%), p=0.0017. From this we determined that *A. membranaceus* did not have an effect on the amount of beads phagocytosed, instead time was the main factor (fig 2).

**Discussion**

From the results it is apparent that *A. membranaceus* has a significant effect on the overall rate of phagocytosis in IC-21 macrophages. When the macrophages
were subjected to *A. membranaceus* there was a 50% increase in the rate of phagocytosis. There was no significant effect of ethanol on the macrophages; therefore we can attribute the increase in the rate of phagocytosis to *A. membranaceus*. It seems that time has no effect on the rate of phagocytosis of macrophages since the rates of phagocytosis were similar for both 60 and 90 minutes. *A. membranaceus* does not have an effect on the amount of beads that each macrophage phagocytosed. However, time does have an effect on how many beads the macrophages phagocytose.

From this we can determine that *A. membranaceus* does have immuno-stimulating properties. *A. membranaceus* has the ability to increase the rate of phagocytosis in macrophages in vitro which could potentially increase the activation of the acquired immune response, antibody production and interleukin secretion. This information could be beneficial to people who have been immuno-compromised and are lacking essential immune functions.

To fully understand *A. membranaceus* and its immuno-stimulating properties further research is needed. The study only showed its effect on *in vitro* macrophages which does not take into account the metabolic processes of *in vivo* studies supplements must overcome. With further research the compounds in *A. membranaceus* that are responsible for its immuno-stimulating properties could be identified, extracted and purified to be used in future pharmacological drugs.
Acknowledgements

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Figures

**Figure 1** - Rate of phagocytosis of IC-21 macrophages

**Figure 2** - Rate of phagocytosis of IC-21 macrophages of more than one bead.
References

1- Sakurai, Matsumoto, Kiyohara, Yamada (1999) “B-cell proliferation activity of pectin polysaccharide from a medicinal herb, the roots of *Bupleurum falcatum* and its structural requirement” *Immunology* 97 (3), 540–547

