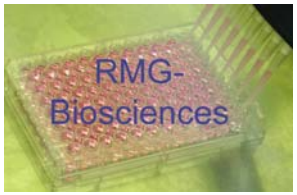


# Immulina, A Spirulina-derived High Molecular Weight Polysaccharide, Enhances Chemokine Expression In THP-1 Monocytes

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## ABSTRACT

Spirulina is a dietary supplement valued for its immune-enhancing properties. We reported previously that the immunostimulatory effect of Spirulina is associated with a high molecular weight polysaccharide fraction labeled as Immulina. In this study, we evaluated the effect of Immulina on genes encoding the chemokines IL-8, MCP-1, MIP-1α, MIP-1β, IP-10, the cytokines TNF-α, IL-1β, and the enzyme cyclooxygenase-2. THP-1 cells were exposed to various concentrations of Immulina ranging from 1 ng/ml to 100 μg/ml and changes in gene expression were assessed by RT-PCR. For comparison, THP-1 cells were activated with TNF-α, IL-1β or lipopolysaccharide using the same assay conditions. To assess the response of THP-1 cells to Immulina at the protein level, we probed culture supernatants using a cytokine array immunoblot assay. Immulina potently and dose-dependently increased the expression of all five chemokines tested and the expression of TNF-α, IL-1β and COX-2. The immunoblot assays revealed a significant increase in the chemokines IL-8 and MIP-1β. Thymidine uptake experiments verified that Immulina did not affect the viability and growth rate of THP-1 cells. The results demonstrate that the immunostimulatory properties of Immulina can be linked to its ability to activate inducible chemokines in cells of the monocyte/macrophage system.

## INTRODUCTION

Spirulina is a member of the blue-green alga family that is attracting interest as a dietary supplement. Recently, Pugh et al. (2001) showed that the immune stimulatory activity of *Spirulina platensis* can be traced to water soluble high molecular weight polysaccharides. This fraction, labeled Immulina, activates the nuclear factor-κB (NF- κB) signaling pathway in human monocytic THP-1 cells. NF- κB regulates the expression of numerous genes involved in the initiation of the inflammatory response, including cytokines, chemokines and adhesion molecules. Pugh et al. (2001) previously reported that Immulina potently stimulates the expression of genes encoding the pro-inflammatory cytokines tumor necrosis factor-alpha (TNF-α) and interkeukin-1β (IL-1β) and chemokines. In this study, we monitored the effect of Immulina on the expression of genes encoding the chemokines IL-8, monocyte chemotactic protein-1 (MCP-1), macrophage inflammatory protein-1 (MIP-1), interferon-γ inducible protein-10 (IP-10). THP-1 cells were exposed to concentration of Immulina ranging from 1 ng/ml to 100 μg/ml and changes in gene expression were monitored by RT-PCR. For comparison, THP-1 cells were activated with 1 ng/ml TNF-α, 10 ng/ml IL-1β, or 10 ng/ml of lipopolysaccharide (LPS) using the same assay conditions. IL-1β gene induction was also determined by real-time RT-PCR. To assess the response of THP-1 cells to Immulina at the protein level, culture supernatants were probed using a cytokine array immunoblot assay. The data revealed that Immulina dose-dependently increased the expression of all five chemokines tested as well as the expression of TNF-α, IL-1β, and cyclooxygenase-2 (COX-2). The cytokine array immunoblot assay revealed an increase in IL-8 and MIP-1β protein. The results of the experiments confirm that Immulina is an activator of the monocyte/macrophage system capable of stimulating the recruitment of diverse populations of leukocytes in response to inflammatory signals.

## METHODS

The human macrophage cell line THP-1 were used for analysis. Cells were seeded (5 x $10^5$ / well) in 6-well plates and incubated for 24 hrs at 37ºC and 5% $CO_2$ before adding: (a) negative control medium alone, (b) Immulina or (c) positive control activators TNF-α (1 ng/ml), IL-1β (10 ng/ml) or LPS (10 ng/ml). Plates were then incubated for 1 or 24 hrs. For RT-PCR experiments, mRNA was collected after 1 hour incubation and measured by semiquantitative RT-PCR. Real-time RT-PCR was used to verify the results for IL-1β mRNA. Cytokine production was determined in culture supernatants after 24 hour incubation using a RayBiotech cytokine antibody microarray. To test for potential cytotoxicity, THP-1 cells were incubated with varying amounts of Immulina for 24 hours and cell proliferation was determined by incorporation of tritiated thymidine.

## RESULTS

- Exposure of THP-1 cells to Immulina for 1 hour produced a dose-dependent increase in mRNA levels of TNF-α, IL-1β, and COX-2 (Figures 1 and 2). These increases were detectable at doses as low as 1μg/ml.
- RT-PCR analysis showed that Immulina dose-dependently induced the expression of the chemokines IL-8, MCP-1, MIP-1α, MIP-1β and IP-10 (Figure 3).
- Cytokine protein array analysis revealed that Immulina induced the secretion of IL-8 and MIP-1β protein from THP-1 cells (Figure 4). Measurements of DNA synthesis by tritiated thymidine uptake showed that Immulina did not affect the viability and rate of proliferation of THP-1 cells at any of the doses tested (Figure 5).

**Fig. 1**:

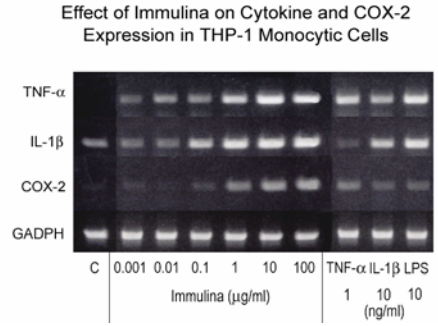



**Fig. 2:** Effect of Immulina on IL-1β mRNA determined by real-time RT-PCR

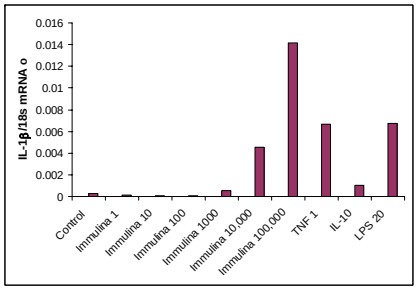



**Fig. 3**:

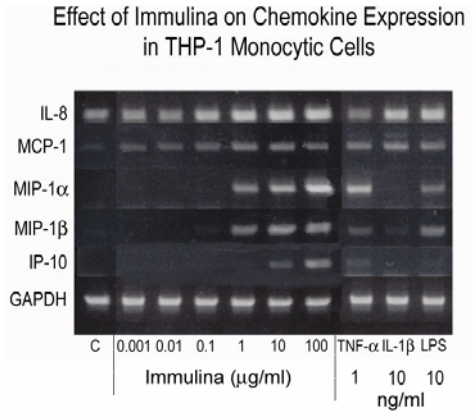



**Fig. 4**: Human cytokine protein array assay (RayBio Human Inflammation Antibody Array III). 1 = standard, 2 = IL-1β, 3 = IL-8, 4 = MIP-1β.

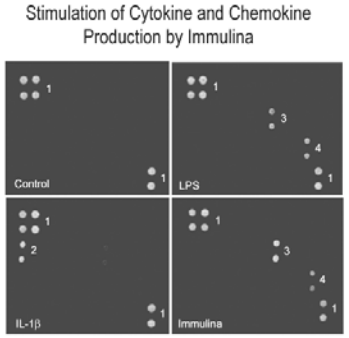
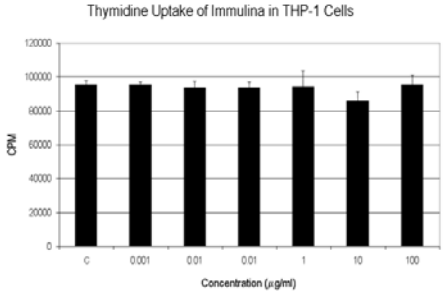



**Fig. 5**:




## CONCLUSIONS

Immulina is a high molecular weight polysaccharide fraction isolated from Spirulina. It potently stimulates the expression of an array of genes encoding pro-inflammatory cytokines and chemokines in THP-1 cells. The result demonstrate that the high molecular weight polysaccharides contained in Spirulina are a key contributor to the immune-enhancing properties of this popular dietary supplement. *In vitro* cytotoxicity assays showed that Immulina at doses up to 100 μg/ml did not affect the cell viability nor the proliferative capacity of THP-1 cells indicating Immulina, like Spirulina, has a high safety margin. Polysaccharides of microbial and plant origin have previously been recognized as immunostimulants. The effect of Immulina on genes encoding chemokines described in this study suggests that this preparation is capable of stimulating cells of the macrophage/monocyte system. Further characterization of Immulina may provide a well defined preparation for the therapy of bacterial and viral infections.

## REFERENCES

Pugh et al. (2001) Planta Medica 67: 737-742

## ACKNOWLEDGEMENTS

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