Quality Control of Polysaccharides from Chinese Medicines

Based on Immunomodulatory Activity

by

MENG Lan-Zhen

Doctor of Philosophy in Biomedical Sciences

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Institute of Chinese Medical Sciences
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SUPERVISOR: Professor LI Shao-Ping

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endless patience and support during these years.
Abstract

Polysaccharides, one kind of main active compounds in Chinese medicines (CM), can be used as markers for quality control of CM. It is generally believed that biological activities of polysaccharides are closely related to their physico-chemical and/or structural properties. Unfortunately, chemical analysis of polysaccharides is a great challenge. However, various chemical characteristics previously found in polysaccharides from different species of *Dendrobium*, *Ganoderma* and *Cordyceps* were correlated with their immunomodulation. Therefore, a comparison of immunomodulatory activities of polysaccharides from CM is helpful to elucidate their efficacies and understand their quality.

In this thesis, the effects of polysaccharides from CM on RAW 264.7 murine macrophage functions, such as phagocytosis, release of NO and cytokines IL-1α, IL-6, IL-10 and TNF-α, were investigated and compared. The results showed that the effects of polysaccharides from different species of *Dendrobium* and *Ganoderma* on macrophages varied. Even polysaccharides in the same CM but collected from different places displayed variant activities, e.g. polysaccharides in *D. officinale* collected from Yunnan Province exerted the strongest immunomodulatory activities. Chemical characteristic studies showed that polysaccharides from *Dendrobium* and *Ganoderma* were diverse. This might contribute to their various effects. The cultured *Cordyceps* mycelia based on different fungi variously up-regulated macrophage functions, which mainly attribute to the effects of polysaccharides. Polysaccharides from cultured UM01 mycelia especially showed the strongest stimulating activities on macrophages.

It was interesting to find that UM01 polysaccharides induced macrophages
differentiated into dendritic-like cells. They induced dendritic cells maturation as demonstrated by increasing expression of antigen-presenting (MHC II), costimulatory (CD80 and CD86) molecules and production of IL-1α/β, IL-6 and TNF-α, decreasing antigen capture capacity and enhancing allogenic T cell stimulation. Besides, UM01 polysaccharides triggered IFN-γ and TNF-α production from human NK cells. The mechanism underlying the macrophage regulation of UM01 polysaccharides might be related to the activation of MAPK and NF-κB signaling pathways. These results suggested that cultured UM01 mycelia could be explored as a novel functional food.

In conclusion, diverse polysaccharides in CM led to their different immunomodulation. Study on immunomodulatory activity was an alternative method for quality evaluation of CM based on the polysaccharides.
Declaration

I declare that the thesis here submitted is original except for the source materials explicitly acknowledged and that this thesis as a whole, or any part of this thesis has not been previously submitted for the same degree or for a different degree.

I also acknowledge that I have read and understood the Rules on Handling Student Academic Dishonesty and the Regulations of the Student Discipline of the University of Macau.
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<th>Full Form</th>
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<tr>
<td>AE</td>
<td>aqueous extract</td>
</tr>
<tr>
<td>ANOVA</td>
<td>one-way analysis of variance</td>
</tr>
<tr>
<td>BSA</td>
<td>bovine serum albumin</td>
</tr>
<tr>
<td>CFSE</td>
<td>5(6)-Carboxyfluorescein diacetate N-succinimidyl ester</td>
</tr>
<tr>
<td>CI</td>
<td>cell index</td>
</tr>
<tr>
<td>CM</td>
<td>Chinese medicines</td>
</tr>
<tr>
<td>CPs</td>
<td>crude polysaccharides</td>
</tr>
<tr>
<td>DC</td>
<td>dendritic cell</td>
</tr>
<tr>
<td>DMEM</td>
<td>Dulbecco’s Modified Eagle Medium</td>
</tr>
<tr>
<td>DMSO</td>
<td>dimethyl sulfoxide</td>
</tr>
<tr>
<td>FBS</td>
<td>fetal bovine serum</td>
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<tr>
<td>GC</td>
<td>gas chromatography</td>
</tr>
<tr>
<td>GL</td>
<td><em>Ganoderma lucidum</em></td>
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<tr>
<td>GS</td>
<td><em>Ganoderma sinense</em></td>
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<tr>
<td>HPLC</td>
<td>high-performance liquid chromatography</td>
</tr>
<tr>
<td>HPSEC-ELSD</td>
<td>high performance size exclusion chromatography-evaporative light scattering detection</td>
</tr>
<tr>
<td>HPTLC</td>
<td>high-performance thin-layer chromatography</td>
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<tr>
<td>IL</td>
<td>interleukin</td>
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<tr>
<td>iNOS</td>
<td>inducible nitric oxide synthase</td>
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<tr>
<td>IP-10</td>
<td>IFN-gamma-inducible protein 10</td>
</tr>
<tr>
<td>KC</td>
<td>keratinocyte-derived chemokine</td>
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<tr>
<td>LPS</td>
<td>lipopolysaccharide</td>
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<tr>
<td>MALLS</td>
<td>multi-angle laser light scattering</td>
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<td>MCP-1</td>
<td>monocyte chemotactic protein-1</td>
</tr>
<tr>
<td>MFI</td>
<td>mean fluorescence intensity</td>
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<tr>
<td>MHC</td>
<td>major histocompatibility complex</td>
</tr>
<tr>
<td>MIP-1α</td>
<td>macrophage inflammatory protein-1α</td>
</tr>
<tr>
<td>MS</td>
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<tr>
<td>MTT</td>
<td>3-(4, 5-dimethylthiazol-2-yl)-2, 5-diphenyl tetrazolium bromide</td>
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<tr>
<td>MLR</td>
<td>mixed lymphocyte reaction</td>
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<tr>
<td>Acronym</td>
<td>Full Form</td>
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<tr>
<td>---------</td>
<td>-----------</td>
</tr>
<tr>
<td>MW</td>
<td>molecular weight</td>
</tr>
<tr>
<td>NF-κB</td>
<td>Nuclear factor-kappa B</td>
</tr>
<tr>
<td>NO</td>
<td>nitric oxide</td>
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<tr>
<td>PBMCs</td>
<td>peripheral blood mononuclear cells</td>
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<td>PBS</td>
<td>phosphate buffered saline</td>
</tr>
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<td>PI</td>
<td>propidium iodide</td>
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<td>PolyB</td>
<td>polymyxin B</td>
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<tr>
<td>RT-PCR</td>
<td>real time quantitative PCR</td>
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<tr>
<td>RI</td>
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<td>SM</td>
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