ABSTRACT

Polysaccharides isolated from various medicinal mushrooms and mycelia are known to activate non-specific immune responses in the host and are now an upcoming source of treatment against cancer and other microbial diseases. However, knowledge pertaining to structure-function relationship and intertwining of signaling pathways induced by these polymeric molecules still remains obscure. This thesis thus focuses on establishing a correlation between structure and immune function of a novel heteroglycan by assessing the functional efficacies of its soluble and particulate forms and also deciphering the underlying molecular crosstalks. A novel water soluble heteroglycan of ~2700 kDa was isolated from the mycelia of an edible mushroom Pleurotus ostreatus and through IR, NMR and GLC-MS studies, was found to be composed of glucose, mannose and fucose in a 3:2:1 ratio with (1→2), (1→3), (1→4) and (1→6) linkages. Studies to assess immunomodulatory activity were conducted in RAW 264.7, a murine macrophage cell line and also in several tumor induced mice models in which the heteroglycan showed increased splenocyte proliferation, NO production, cytokine (TNF-α, IFN-γ) secretion and tumor inhibition along with many other functions. Effectiveness of the heteroglycan was also assessed in a three dimensional tumor spheroid and macrophage co-culture milieu wherein the results were in sync with the data from mice experiments corroborating that this system may serve as a befitting replacement of tedious animal-based experiments. The heteroglycan was then chemically conjugated with biocompatible dendrimers or nanoparticles to achieve a particulate form that exhibited two fold higher immune response than that of the soluble unconjugated form, implying that a multivalent structure possibly enhances immunostimulation by engaging more glycan binding receptors on immune cells. Furthermore, acid hydrolysed heteroglycan fraction of at least 10-30 kDa elicited prominent immune response when its size was increased by conjugation with dendrimer, signifying that size is critical for receptor activation and the smallest binding unit of the heteroglycan that can trigger an immune response, lies in the 10-30 kDa range. The immune response was found to be mediated by receptors such as Dectin-1 and TLR2 which caused the downstream phosphorylation of MEK, p38 and ERK1/2, followed by the nuclear translocation of NF-κβ transcription factor and the subsequent expression of a gamut of cytokines. To conclude, these findings strengthen the growing evidence of the immunomodulatory potential of glycans and advocate their structure-function relationship which may serve as important inclusions to the armoury of strategies employed for immune based therapies.

Keywords: *Pleurotus ostreatus*, Mycelia, Heteroglycan, Immunomodulatory, Tumor inhibition, Tumor spheroid, Particulate heteroglycan, Macrophages, Immune based therapies.