Exploring TLR-Mediated Modulation of Protein Glycosylation and Sialylation in THP-1 Cells

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Toll-like receptors (TLRs) play a crucial role in the innate immune system, serving as the first line of defense against invading pathogens. These pattern recognition receptors (PRR) can detect conserved molecular patterns found in various microorganisms, known as pathogen-associated molecular patterns (PAMPs). TLRs recognize a wide array of microbial components, including bacterial lipopolysaccharides (LPS), viral RNA, and fungal polysaccharides. There are different types of TLRs, each with distinct recognition specificities and intracellular responses. For example, TLR4 primarily recognizes bacterial LPS, TLR3 detects double-stranded RNA (a viral replication intermediate), and TLR9 is activated by unmethylated CpG DNA, commonly found in bacterial and viral genomes. While the intracellular changes that occur upon TLR activation are well-studied, questions remain about how this process affects crucial cellular signaling pathways, such as protein glycosylation and sialylation. Our group used LPS and Poly(I:C) to investigate whether the activation of different types of TLRs activates protein glycosylation pathway. We used THP-1 cell line differentiated with 5 ng/mL PMA as our study model, and for comparisons, we stimulated the cells with 15 ng/mL of LPS or 25 ng/mL of Poly(I:C) for 24 hours. We utilized various techniques to investigate glycosylation patterns, including quantitative proteomics, mass spectrometry-based glycosylation profiling, as well as sialic acid quantification by HPLC-FLD, lectin blotting, and PCR. Our results from the HPLC-FLD showed a significant difference in the presence of sialic acids in proteins from cells stimulated with LPS compared to the control and Poly(I:C) groups. This finding was corroborated by lectin blotting, where we observed the same pattern of sialylation. The quantitative proteomics revealed a notable difference in the proteins identified in the control group compared to the tested groups. We identified one OST protein complex subunit, a key enzyme in the protein glycosylation pathway, upregulated in cells treated with both LPS and Poly(I:C). Additionally, we observed an increase in proteins involved in vesicular trafficking, such as Sec22b, an important protein for ER-Golgi protein trafficking. Our work utilizes a wide range of techniques to provide an initial overview of changes in protein glycosylation when different TLR pathways are activated. This offers new perspectives for future investigations aimed at linking innate immunity and protein glycosylation processes.

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