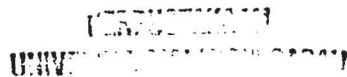


**GLYCOFORMS AND IMMUNOSUPPRESSIVE
ACTIVITIES OF TAMM-HORSFALL
GLYCOPROTEIN AND UROMODULIN**

HONG CHIA YEAN



**THESIS SUBMITTED IN FULFILLMENT FOR
THE DEGREE OF MASTER OF SCIENCE**

**SCHOOL OF SCIENCE AND TECHNOLOGY
UNIVERSITI MALAYSIA SABAH
2013**



UMS
UNIVERSITI MALAYSIA SABAH

ABSTRACT

GLYCOFORMS AND IMMUNOSUPPRESSIVE ACTIVITIES OF TAMM-HORSFALL GLYCOPROTEIN AND UROMODULIN

Tamm-Horsfall glycoprotein (THP) and uromodulin are the most abundant glycoproteins in the urine of non-pregnant women and pregnant women, respectively. Today, the structural profile and biological activities of these glycoproteins are controversial. This study was to elucidate the glycan profiles of THP and uromodulin, to investigate the immunosuppressive activities of these glycoproteins and to correlate the pregnancy-associated changes to their immunological functionality. A higher protein purity of THP and uromodulin were isolated from diatomaceous earth filtration. MALDI-TOF/TOF MS analysis showed that both THP and uromodulin expressed high mannose and complex-type *N*-glycan carrying sialic acid residues, Sd^a, LacNAc and LacdiNAc sequence as the capping antennae. But uromodulin tended to express multiple degree of sialylation, in which up to four sialic acid residues were expressed on its tetra-antennary *N*-glycans. High-mannose glycan, the Man₆GlcNAc₂ of uromodulin was the most abundant signal among all its *N*-glycans. Core 1 and Core 2-type *O*-glycans were observed in THP and uromodulin, with THP expressing more *O*-glycans. MTS viability assay showed that 125 µg/ml of uromodulin and 250 µg/ml of THP significantly reduced the PHA-activated PBMC cell viability, to 85.64 ± 5.92% and 84.59 ± 2.94%, respectively. Comparing to THP, uromodulin was 2-fold more active in suppressing PBMC cell viability. Immunophenotyping analysis showed that CD4⁺ T helper cells, CD8⁺ cytotoxic T cells, CD56⁺ NK cells and CD14⁺ monocytes were suppressed by THP. It induced pro-inflammatory cytokines such as IL-1β, TNF and Th1 cytokine IFN-γ. On the other hand, uromodulin had slightly greater effect in suppressing CD4⁺ T helper cells and CD8⁺ cytotoxic T cells if compared to that of THP. It only induced IL-1β and suppressed both Th1 cytokine IFN-γ and Th2 cytokine IL-10. This indicates that THP has dual-immunomodulatory properties, by expressing both suppressive (on CD4⁺ and CD8⁺ T cells) and stimulatory (on pro-inflammatory cytokines IL-1β and TNF) activities, whereas uromodulin is more immunosuppressive than THP because it suppressed CD4⁺ and CD8⁺ T cells, stimulated IL-1β only and withheld the TNF secretion. In summary, glycosylation changes in uromodulin were shown in this study as compared with THP, which might contribute to its immunosuppressive property.