Sample Abstract

Quantitative PCR Analysis of Mouse Toll-Like Receptors

Cerrone Foster and Dr. John Laffan, Department of Microbiology, College of Medicine, East Tennessee State University, Johnson City, TN.

The immune system is a complex and varied defense mechanism used to fight disease and infection. One way the body recognizes infection is through recognition of Pathogen Associated Molecular Patterns (PAMPs). Two known PAMPs, lipopolysaccharide (LPS) and glucan, are microbial products that can activate the immune system. However, the intracellular signaling pathways of the immune system are not clearly defined. It has recently been found that Toll-like receptors (TLRs) are involved in this signaling process. Stimulation of these receptors by PAMPs can initiate a signaling cascade, resulting in activation of genes needed to illicit an immune response. We therefore investigated the quantitative regulation of TLR2 and TLR4 in the presence of LPS and glucan. Using a mouse macrophage cell line (J774a.1 cells), LPS and glucan were added (1 ug/ml) to the cells or equal volume of carrier was added as a control. RNA was isolated at 1,4, and 24 hour time intervals. The RNA as reversed transcribed using a oligo dT primer and that cDNA was quantified using Quantitative PCR. Primer sets specific for TLR2 and TLR4 were designed and the reactions were run in a BioRad iCylcer real-time PCR machine. In the presence of LPS, TLR2 and TLR4 decreased during the early time intervals and dramatically increased at the 24-hour interval. In the presence of glucan, there was no significant change in TLR2 and TLR4 mRNA over time. Results of this work identified an early down regulation as well as late up regulation of TLR2 and TLR4 mRNA in the presence of LPS. This work will be a useful tool in understanding the roles of TLR2 and TLR4 in the immune response. Understanding the role of these TLRs during immune response can lead to the development of novel drugs to treat disease and infection.