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Production of tumor necrosis factor (TNF) from endotoxin-stimulated macrophages and its regulation

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Tumor necrosis factor (TNF) is mainly produced by activated T lymphocytes, monocytes, and macrophages and plays critical roles in the regulation of immune responses against infectious agents. Inappropriate production of TNF is implicated in many pathologic conditions such as septic shock, cerebral malaria, rheumatoid arthritis, and multiple sclerosis. The synthesis of TNF is known to be regulated at transcriptional, translational, and post-translational levels.

Serine proteinase inhibitors such as N-tosyl-L-phenylalanine chloromethyl ketone (TPCK) and *Nα-p*-tosyl-L-lysine chloromethyl ketone (TLCK) were shown to inhibit production of TNF in lipopolysaccharide (LPS)-activated RAW 264.7 macrophages. The proteinase inhibitors were also reported to inhibit activation of transcription factor NF-κB by blocking signaling pathway for stimuli-induced phosphorylation of inhibitory subunit (IκBα) and thus preventing its degradation. In RAW 264.7 cells TPCK and TLCK significantly suppressed LPS-induced increase in TNF mRNA, induction of nuclear κB-binding activity and degradation of IκBα. TPCK and TLCK effectively blocked TNF mRNA synthesis even when they were added after LPS stimulation. In these cells, however, inhibitory modes of two inhibitors were found to be different: while addition of TLCK suppressed IκBα degradation and reduced NF-κB activity, comparable decrease in the nuclear κB-binding activity or IκBα degradation was not observed in cells treated with TPCK. Our results show that TPCK inhibits LPS-induced TNF mRNA synthesis in the presence of activated NF-κB and suggest that mechanisms other than NF-κB activation are involved in the transcriptional regulation of TNF gene.