Inhibition of LPS-induced inflammatory cytokine expression with a new immunosuppressive compound

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Background:

A controlled inflammatory response to infection or injury can be beneficial, while an exacerbated or prolonged response can be detrimental. Tryptophan 2,3-dioxygenase (TDO), a rate-limiting enzyme of the kynurenine pathway of tryptophan metabolism, has been reported to link between immune responses and inflammatory diseases such as cancer, sepsis and depression. In this study, we examined the effects of a TDO inhibitor, compound X, on the expression of LPS-stimulated pro-inflammatory cytokines in a mouse macrophage cell line, Raw 264.7, and primary peritoneal macrophages prepared from wild-type and TDO knockout mice.

Methods:

Primary peritoneal macrophages were prepared from thioglycollate-elicited peritoneal cells in male wild-type and TDO knockout mice. Primary peritoneal macrophages and Raw264.7 cells were treated with 100 ng/ml LPS for 5 h with or without compound X (1 or 10 μ M). Total RNA was purified from macrophages and Raw264.7 cells using TRIzol reagent. The expression of IL-1 β , IL-6 and TNF- α mRNA was determined by qRT-PCR. To determine mechanisms by which compound X regulates LPS-induced cytokine expression, the phosphorylation of ERK, Akt, p70S6 kinase (p70S6K), and I κ B α in Raw264.7 cells were analyzed by Western blotting.

Results:

In Raw264.7 cells, compound X attenuated LPS-induced mRNA expression of IL-1 β and IL-6, but not TNF- α . Compound X also attenuated LPS-induced mRNA expression of IL-1 β and IL-6 in primary peritoneal macrophages prepared from wild-type mice. Interestingly, the inhibitory effects of compound X on LPS-induced mRNA expression of IL-1 β and IL-6 were observed in primary peritoneal macrophages prepared from TDO knockout mice. LPS strongly increased the phosphorylation levels of ERK, Akt (Ser473 and Thr308), p70S6K (Thr389) and I κ B α (Ser32/36) in Raw264.7 cells. Treatment of Raw264.7 cells with compound X inhibited LPS-induced phosphorylation of Akt and p70S6K, but not ERK or I κ B α .

Conclusion:

These observations suggest that compound X likely acts as an inhibitor of LPS-induced inflammatory responses via mechanisms involving the suppression of Akt and p70S6K signaling.