

**[S-1]****Potential of COX-2 Induction by C2-ceramide,  
a Potential Cell Death Marker**

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Ceramide, a potential cell death marker formed by sphingomyelinase, is involved in the expression of cyclooxygenase-2 (COX-2). This study examines the effect of C2-ceramide (C2), a cell-permeable ceramide analog, on the LPS-inducible COX-2 expression and signaling pathways. C2 did not induce COX-2, but potentiated LPS-inducible COX-2 expression in Raw264.7 cells, whereas dihydro-C2 was inactive. Treatment of cells with C2 notably increased LPS-inducible CCAAT/enhancer binding protein (C/EBP) DNA binding. Antibody supershift experiments revealed that LPS-induced C/EBP DNA binding activity depended on C/EBP $\beta$  and C/EBP $\delta$ , but not C/EBP $\alpha$ , C/EBP or CBP/p300. C/EBP $\beta$  contributed to C2-enhanced DNA binding activity. SB203580, a p38 kinase inhibitor, completely inhibited LPS-inducible and C2-potentiated LPS-inducible COX-2 expression. Enhancement of LPS-inducible COX-2 expression and C/EBP DNA binding by C2 was abrogated in dominant negative mutant of JNK1 [JNK1(-)] cells. PD98059 or stable transfection with dominant negative mutant of MKK1 [MKK1(-)] decreased COX-2 induction by LPS, but failed to inhibit C2-enhanced LPS induction of COX-2. Transfection with dominant-negative mutant of C/EBP inhibited the ability of C2 to potentiate the induction of COX-2 by LPS. In LPS-treated cells, C2 enhanced both the nuclear translocation and the expression of LPS-inducible C/EBP $\beta$  with an increase in AP-1 DNA binding activity. These enhancements were abolished by JNK1(-) transfection. AP-1 decoy oligonucleotide suppressed C2-potentiated C/EBP $\beta$  expression, indicating that AP-1 was responsible for

C2-mediated C/EBP $\beta$  expression. These results demonstrate that C2 increases C/EBP $\beta$ -mediated COX-2 induction by LPS and that the pathway of JNK1, but not ERK1/2, is responsible for C/EBP $\beta$  activation involving AP-1-mediated enhanced C/EBP $\beta$  expression.