ABSTRACT

Exposure to cigarette smoke extract (CSE) leads to airway and lung inflammation through an oxidant-antioxidant imbalance. Cyclooxygenase-2 (COX-2) and prostaglandin E_2 (PGE_2) have been shown to play critical roles in respiratory inflammation. Here, we show that COX-2/PGE_2/IL-6 induction is dependent on Toll-like receptor 4 (TLR4)/NADPH oxidase signaling in human tracheal smooth muscle cells (HTSMCs). CSE induced COX-2 expression in vitro in HTSMCs and in vivo in the airways of mice. CSE also directly caused an increase in TLR4. Moreover, CSE-regulated COX-2, PGE_2, and IL-6 generation was inhibited by pretreatment with TLR4 Ab; inhibitors of c-Src (PP1), NADPH oxidase (diphenylene iodonium chloride and apocynin), p38 MAPK (SB202190), MEK1/2 (U0126), JNK1/2 (SP600125), and NF-κB (helenalin); a ROS scavenger (*N*-acetyl-L-cysteine); and transfection with siRNA of TLR4, MyD88, TRAF6, Src, $p47^{phox}$, p38, p42, JNK2, or p65. CSE-induced leukocyte numbers in BAL fluid were also reduced by pretreatment with these inhibitors. Furthermore, CSE induced $p47^{phox}$ translocation and TLR4/MyD88/TRAF6 and c-Src/$p47^{phox}$ complex formation. We found that PGE_2 enhanced IL-6 production in HTSMCs and leukocyte count in BAL fluid. In addition, treatment with nicotine could induce COX-2, PGE_2, and IL-6 generation in in vivo and in vitro studies. These results demonstrate that CSE-induced ROS generation was mediated through the TLR4/MyD88/TRAF6/c-Src/NADPH oxidase pathway, in turn initiated the activation of MAPKs and NF-κB, and ultimately induced COX-2/PGE_2/IL-6-dependent airway inflammation.