Heme oxygenase (HO)-1 is a stress-inducible rate-limiting enzyme in heme degradation that confers cytoprotection against oxidative injury and provides a vital function in maintaining tissue homeostasis. Increasing reports have indicated that lipoteichoic acid (LTA) exerts as LPS as an immune system-stimulating agent and plays a role in the pathogenesis of severe inflammatory responses induced by Gram-positive bacterial infection. We report that LTA is an inducer of HO-1 expression mediated through the signaling pathways in human tracheal smooth muscle cells (HTSMCs). LTA-induced HO-1 protein levels, mRNA expression, and promoter activity were attenuated by transfection with dominant negative mutants of TLR2 and MyD88, by pretreatment with the inhibitors of c-Src (PP1), NADPH oxidase (diphenylene iodonium chloride (DPI) and apocynin (APO)), and reactive oxygen species (ROS) scavenger (*N*-acetyl-L-cysteine) or by transfection with small interfering RNAs of Src and NF-E2-related factor 2 (Nrf2). LTA-stimulated translocation of $p47^{phox}$ and Nrf2 or ROS production was attenuated by transfection with dominant negative mutants of TLR2, MyD88, and c-Src and by pretreatment with DPI or APO. Furthermore, LTA-induced TLR2, MyD88, TNFR-associated factor (TRAF)6, c-Src, and $p47^{phox}$ complex formation was revealed by immunoprecipitation using an anti-TLR2 or anti-c-Src Ab followed by Western blot analysis against an anti-TLR2, anti-MyD88, anti-TRAF6, anti-c-Src, or anti-$p47^{phox}$ Ab. These results demonstrated that LTA-induced ROS generation was mediated through the TLR2/MyD88/TRAF6/c-Src/NADPH oxidase pathway, in turn initiates the activation of Nrf2, and ultimately induces HO-1 expression in HTSMCs. *The Journal of Immunology*, 2008, 181: 5098–5110.