Occludin is one of the key tight junction (TJ) proteins in endothelial cells and it plays an important role in modulating blood brain barrier (BBB) function. This protein (65kDa) has been shown to engage in many signaling pathways and subjected to phosphorylation by a number of protein kinases. Activation of endothelial cells by pro-inflammatory cytokines and endotoxin (lipopolysaccharides, LPS) may alter TJ proteins and BBB functions. Here we describe the responses of occludin in immortalized human cerebral endothelial cells (hCMEC/D3 cells) stimulated by TNFalpha, IL-1beta and LPS. Exposing cells to TNFalpha resulted in a rapid and transient band shift of occludin suggesting an increase in phosphorylation whereas IL-1beta and LPS produced significantly less effects on the band shift. TNFalpha also caused transient stimulation of p38MAPK and ERK1/2 in hCMEC/D3 cells, and TNFalpha-induced occludin phosphorylation was suppressed by SB202190, inhibitor for p38MAPK. Cells treated with TNFalpha for 24h resulted in cell morphology changes, a decrease in the expression of occludin, and enhanced endothelial permeability, as determined by the FITC-dextran assay and TEER measurement with cells grown in transwell inserts. In addition, TNFalpha-induced reduction of occludin was abrogated by SB202190. Collectively, these data demonstrate effects of TNFalpha on occludin and cerebral endothelial cell function through the activation of p38MAPK pathway.