

P020 Polymorphic variant D299G.T399I of TLR4 exhibits reduced activation by monomeric endotoxin:MD-2 complex
Polonca Prohinar, Theresa Gioannini
and Jerrold Weiss
University of Iowa, Iowa City, IA 52241, USA

Expression of the polymorphic variant D299G.T399I of TLR4 is associated with increased airway hyporesponsiveness to inhaled endotoxin in vivo, increased hyporesponsiveness of primary airway cells in vitro and reduced CD14/MD-2-dependent activation by endotoxin (E). To determine if these discrete structural alterations of the TLR4 ectodomain affect its interaction with monomeric E:MD-2 complex, a potent TLR4 agonist, we measured IL-8 production by HEK293T cells transiently transfected with wt or D299G.T399I TLR4 and incubated \pm increasing concentrations of E:MD-2. Our results demonstrate that, compared to wt TLR4, D299G.T399I TLR4 mediates up to 50% less cell activation by E:MD-2 as measured by IL-8 secretion. Studies are in progress to compare binding of [³H]LOS:MD-2 (25,000 cpm/pmol) to the predicted ectodomain (residues 24-631) of wt and variant TLR4. In summary, our findings indicate that the substitution of Asp 299 with Gly and Thr 399 with Ile alters the interaction of TLR4 with E:MD-2 and may be responsible for the reduced responsiveness of this TLR4 variant to endotoxin. Binding studies in progress should reveal if reduced responsiveness is due to reduced binding of E:MD-2 to TLR4 or reduced activation of TLR4 by bound E:MD-2.