

**P024 MD-2 G56R polymorphic variant shows reduced LPS binding and LPS-dependent activation of TLR4**  
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MD-2, an essential component of LPS sensing, binds LPS independently or in complex with TLR4. Polymorphic variants of the proteins involved in the LPS-recognition cascade such as LBP, CD14 and TLR4 have been previously described. We have investigated two polymorphisms for MD-2, G56R and P157S, observed at a frequency of ~2.5% in Caucasians. As predicted from the MD-2 structural model, the P157S mutation had no effect on LPS responsiveness, while the G56R mutation, which is located close to the LPS-binding region, decreased the LPS response. Both LPS binding (i.e. transfer of LPS from LPS:sCD14 to MD-2) and LPS-dependent activation of HEK/TLR4 cells by soluble MD-2 G56R were much less than that by wild-type MD-2. Co-expression of MD-2 with TLR4 only partially reduced differences in activity of wild-type and G56R MD-2. These findings indicate that the G56R variant of MD-2 has reduced ability to support TLR4-dependent activation by LPS, probably due to reduced transfer of LPS from CD14 to MD-2. Co-expression of wild-type and G56R MD-2 ( $\pm$ TLR4) yielded intermediate MD-2 activity indicating that heterozygotes (MD-2<sup>wt/G56R</sup>) will also have blunted TLR4-dependent responses to LPS and suggesting that TLR4 activation requires engagement of more than one MD-2 molecule.