

P068 Importance of different leucine-rich repeats within TLR9 for activation by CpG-DNA

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Toll-like receptor ectodomains consist of 19-25 leucine-rich repeats (LRR) which together build a solenoid like structure. The common LRR in TLRs is a 24 amino acid motif but there are irregular LRRs containing insertions after position 10 and 15 which are supposed to protrude from the solenoid. TLRs differ in their structure by containing variable numbers of LRRs and by the number and position of the irregular LRRs. TLR7, 8 and 9, all involved in the recognition of nucleic acids, have the same LRR-pattern.

This and studies from other TLRs suggest an involvement of the irregular LRRs and/or their insertions in ligand recognition. Here we try to identify regions important for ligand binding of TLR9 by deleting irregular LRRs or their insertions as well as by switching irregular LRRs from TLR7 to TLR9 and testing the mutants to activation by CpG-DNA and R848. Deletion of any tested LRR or insertion led to irresponsiveness of TLR9 to CpG-DNA. The same was true for the mutant of TLR9 containing one or more LRRs of TLR7. Furthermore, we introduced single amino acid mutations within various LRRs. Disrupting a CXXC-motif, involved in CpG-binding in other proteins, as well as the mutations A219G and D250E, all located in LRR8 led to loss of function. In contrast a corresponding mutation in LRR9 (D293E) had only a marginal effect on receptor function. All mutations so far indicate a pattern confirming the assumed importance of the insertions, especially of that located in LRR8, but further mutations will have to be done to verify this concept.