CS09-4. Molecular mechanism Of poxviral antagonism of the Tlr4 complex by vacv protein A46

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Toll like receptors (TLRs) are involved in the detection of viruses, leading to cytokine and interferon induction. TLR4, although best known for its role in recognising LPS, also plays a role in the immune response to viruses. For example, VSV glycoprotein G induces type I IFN in a TLR4-dependent manner, the induction of proinflammatory cytokines by the F protein of RSV is also TLR4-dependent, and TLR4 is protective in pulmonary vaccinia virus (VACV) infection. Further, a number of viral proteins that interfere with TLR signalling have been identified, thus highlighting the importance of TLRs in anti-viral immunity. We previously showed that the VACV protein A46 inhibits signalling by multiple TLRs and can interact with all known components of the TLR4 complex that contain a TIR domain, i.e. TLR4 itself and the TIR adaptor molecules Mal, MyD88, TRAM and TRIF. This is consistent with the ability of A46 to block multiple signalling pathways emanating from the TLR4 complex, such as LPS-induced MAPK, NFjB and IRF activation. Furthermore, an 11 amino acid peptide derived from A46 termed VIPER (KYSFKLILAEY), when fused to a cell-penetrating delivery sequence, potently and specifically inhibits TLR4 responses. However the exact molecular mechanism whereby A46 disrupts TLR4 signalling remains to be established, and may yield insight into how the TLR4 complex functions. We show that A46 targets the BB loop of TIR proteins and cannot interact with those that do not have a

conserved Proline in their BB loop. Further, A46 acted on TLR4 by disrupting the TLR4-Mal and TLR4-TRAM interactions, but had no effect on the TLR4-TLR4, Mal-MyD88 or TRAM-TRIF interactions. We found that the requirement for a BB loop for protein interface interactions correlated with the protein: protein interfaces antagonised by A46, consistent with the notion that the BB loops are important only in receptor:adapter interactions and not the adapter: adapter or receptor: receptor interactions. When the region of A46 from which VIPER is derived was mutated, the ability of A46 to impair LPS-induced NFjB activation was lost, as was its ability to interact with TRAM. However this mutant still inhibited IL-1R-, TLR2- and TLR8-dependent NFjB activation. Thus VIPER in A46 represents a TLR4-specific inhibitory surface for TRAM antagonism. This study provides the molecular basis for pathogen subversion of TLR4 signalling and clarifies the importance of TIR motif BB loops in the formation of the TLR4 complex