Abstract

MyD88 is one of the key adaptor proteins in the innate immunity and serves as a node connecting activated Toll-like (TLR) and interleukin-1 (IL-1R) receptors with intracellular signalling pathways. Timely and efficient immune response is essential for host defence against invading pathogens. However, the response has to be tightly controlled and regulated as any aberrations can lead to chronic inflammation or cancer. One way to regulate the innate immune responses is to induce expression of alternatively spliced variants with antagonistic activity. MyD88S serves this role in case of MyD88.

After ligand binding/pathogen recognition IL-1R/TLRs undergo dimerization providing a platform for MyD88 binding. This increases local concentration of signalling components which leads to formation of a multi-component structure called myddosome. It is thought that such structures, known as supramolecular organizing centres (SMOCs) serve as second messengers in the innate immune response since they lead to signal propagation and amplification. Myddosome consists of MyD88 and kinases from the IRAK family which undergo phosphorylation and activate downstream proteins. The signal cascade converges on activation of transcription factor NF-κB which induces cytokine production.

MyD88 exhibits a unique domain composition where N-terminal death domain (DD) interacts with IRAK kinases and C-terminal Toll/interleukin-1 receptor (TIR) domain binds with activated receptors. TIR domain can also be found in other adaptor proteins from TLR signalling pathway – e.g., Mal protein. DD and TIR domains in MyD88 are connected with intermediate domain (ID). An alternative splicing variant MyD88S lacks exon2 resulting in an in-frame deletion of ID. MyD88S expression is observed upon prolonged stimulation of TLRs. This alternative splice variant acts as a dominant negative inhibitor of the signalling pathway, does not interact with IRAK kinases and inhibits NF-κB activation. However, a comprehensive mechanistic insight into MyD88S function is needed to explain its dominant negative effect.

The aims of this thesis were to structurally define complexes of TIR_{MyD88} and TIR_{Mal}, identify small molecule inhibitors of this interaction and to elaborate MyD88-ID role in myddosome formation and to gain insight into mechanistic action of inhibitory effect of MyD88S.

First, $TIR_{MyD88}$and TIR_{Mal} were purified to homogeneity. Attempts were made to obtain stable complexes of TIR_{MyD88}- TIR_{Mal} for crystallisation as the interaction of those domains was suggested by literature The formation of stable complexes was not observed. Subsequently, FBDD (fragment-based drug discovery)-NMR was used to identify small molecules that bind to MyD88-TIR and modulate its activity. Relevant small molecule ligands were identified. Selected small molecules were used for crystallization trails to explain the binding mode.

In parallel, detailed structural and functional characterization of ID domain of MyD88 was attempted. Preliminary results concerning individual domain function suggested that ID is essential for signal propagation and alternative splice variant MyD88S does not activate NF-κB. Bioinformatic analysis confirmed that amino acid sequence is highly conserved which may suggest that ID exhibits a defined secondary structure. However, a series of biophysical experiments showed that ID is mostly unstructured, with certain content of transient secondary structures only.

Using alanine scanning it was shown that sequence of only 5 amino acid residues within ID is pivotal for signal propagation. Ala2, a mutant where the 5 resides were mutated to alanines, failed to activate NF-κB (a behaviour comparable to MyD88S). Further investigation identified tyrosine 116 as the only residue within ID essential for signal transduction. Tyr116 participates in a network of intramolecular hydrogen bonds with DDs of adjacent MyD88 molecules stabilizing the myddosome structure

Myddosome formation was investigated in cells using confocal microscopy. It was demonstrated that SMOCs are formed by full length MyD88 (MyD88FL). MyD88S and Ala2 evenly distributed in cells, showing no indication of SMOC formation. These experiments confirmed the assumption that MyD88S is unable to form myddosomes. What is more, when MyD88S was co-expressed with MyD88FL the granular pattern was not observed.

Co-immunoprecipitation experiments indicated that MyD88FL forms dimers with all tested variants (also MyD88S and Ala2). However, only MyD88FL/MyD88FL and not MyD88FL/MyD88S were able to recruit additional MyD88 molecules.

Overall, the results communicated within this thesis demonstrate that ID is largely unstructured despite its high amino acid sequence conservation. 5 consecutive amino acid residues mediate ID function – signal propagation leading to NF-κB activation. Alternative splice variant

MyD88S blocks incorporation of additional MyD88 molecules and thus inhibits myddosome formation. Together these results allow to establish a mechanism for the dominant negative effect of MyD88S.