P29 & FP – **NLRP3 inflammasome is triggered by trimerisation of pyrin domains and can be specifically inhibited by designed peptides**

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NLRP3 inflammasome is a multiprotein complex which forms within cells in response to various stress associated triggers. It consists of oligomerised NLRP3, ASC and procaspase-1 proteins. Caspase-1 activates proinflammatory IL-1β and IL-18 cytokines which drive inflammation through recruitment of neutrophils and other immune cells. Chronic protein or crystal deposits in diseases such as neurodegenerative diseases or atherosclerosis and NLRP3 gene mutations in cryopyrinopathies act as triggers of the NLRP3 inflammasome which contributes to the disease progression.

The mechanism of NLRP3 inflammasome activation including the stoichiometry of NLRP3 oligomerisation is not yet fully elucidated, which hinders the design of effective and specific NLRP3 inflammasome inhibition strategies. Objectives of our research were to determine minimal NLRP3 oligomerisation state necessary to initiate inflammasome complex formation and to design specific peptide based inhibitors of NLRP3 inflammasome.

In order to define the minimal activating NLRP3 oligomerisation state, we prepared retrovirus-transduced stable macrophage cell lines which express $NLRP3^{PYD}$ bound to various oligomerisation domains (dimerisation and trimerisation) under the doxycycline control and observed that trimerisation of NLRP3 was the minimal oligomerisation stoichiometry that promoted inflammasome activation[1].

To inhibit inflammasome formation at different stages we designed a set of putative inhibitory peptides which could potentially interfere with the protein interactions within the complex. The design was based on the crystal structures of the PYD and CARD interaction domains[2] and on the pathological mutation hotspots in the NLRP3 NACHT domain. We identified peptides that were capable of inhibiting the activation of caspase-1 and the release of IL-1β from myeloid cells. Inhibitory effects were observed on macrophages as well as on microglial cells. The inhibitory effect of the peptides was independent of the type of NLRP3 inflammasome trigger. Furthermore, we found that some of the peptides specifically inhibited the NLRP3 inflammasome. Peptides also effectively inhibited inflammasome NLRP3 activation in cell lines with NLRP3 mutations linked to cryopyrinopathies.

We also explored potential applications with one of the identified inhibitory peptides in therapy of neurodegenerative diseases by equipping it with peptide sequence to allow its transfer through the blood-brain barrier. Peptide P3 was localised inside the cells as well as within the brain of mice after intravenous injection showing its potential implications as an NLRP3 inflammasome inhibitor in a neurological setting.

Designed peptides provide an insight into the mechanism of NLRP3 inflammasome assembly. Together with the identified minimal oligomerisation state they provide the basis for the development of novel antiinflammatory strategies.

1 Sušjan P, Roškar S, Hafner-Bratkovič I. 2017. *The mechanism of NLRP3 inflammasome initiation: Trimerization The mechanism of NLRP3 inflammasome initiation: Trimerization but not dimerization of the NLRP3 pyrin domain induces robust activation of IL-1β.* Biochem Biophys Res Commun. 483(2): 823-828.

2 De Alba E. 2009. *Structure and Interdomain Dynamics of Apoptosis-associated Speck-like Protein Containing a CARD (ASC)*. The Journal of Biological Chemistry, 284, 47: 32932-32941