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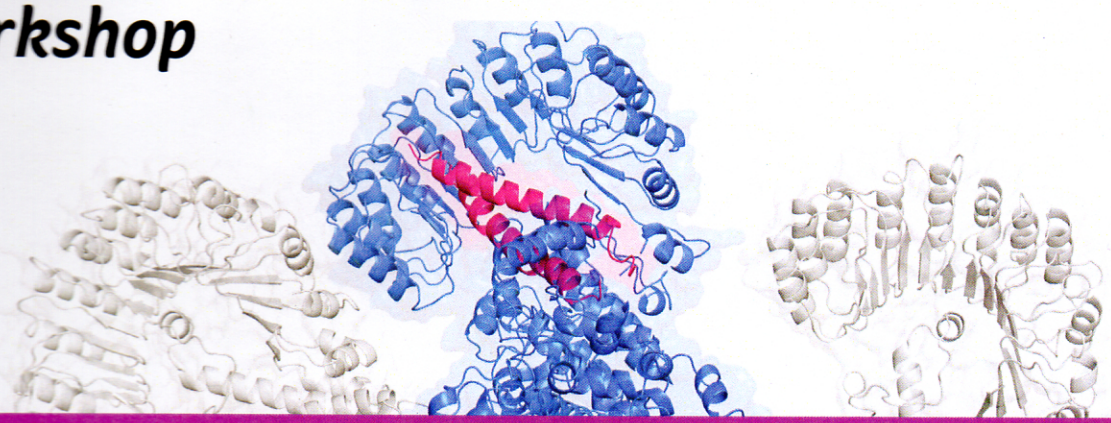
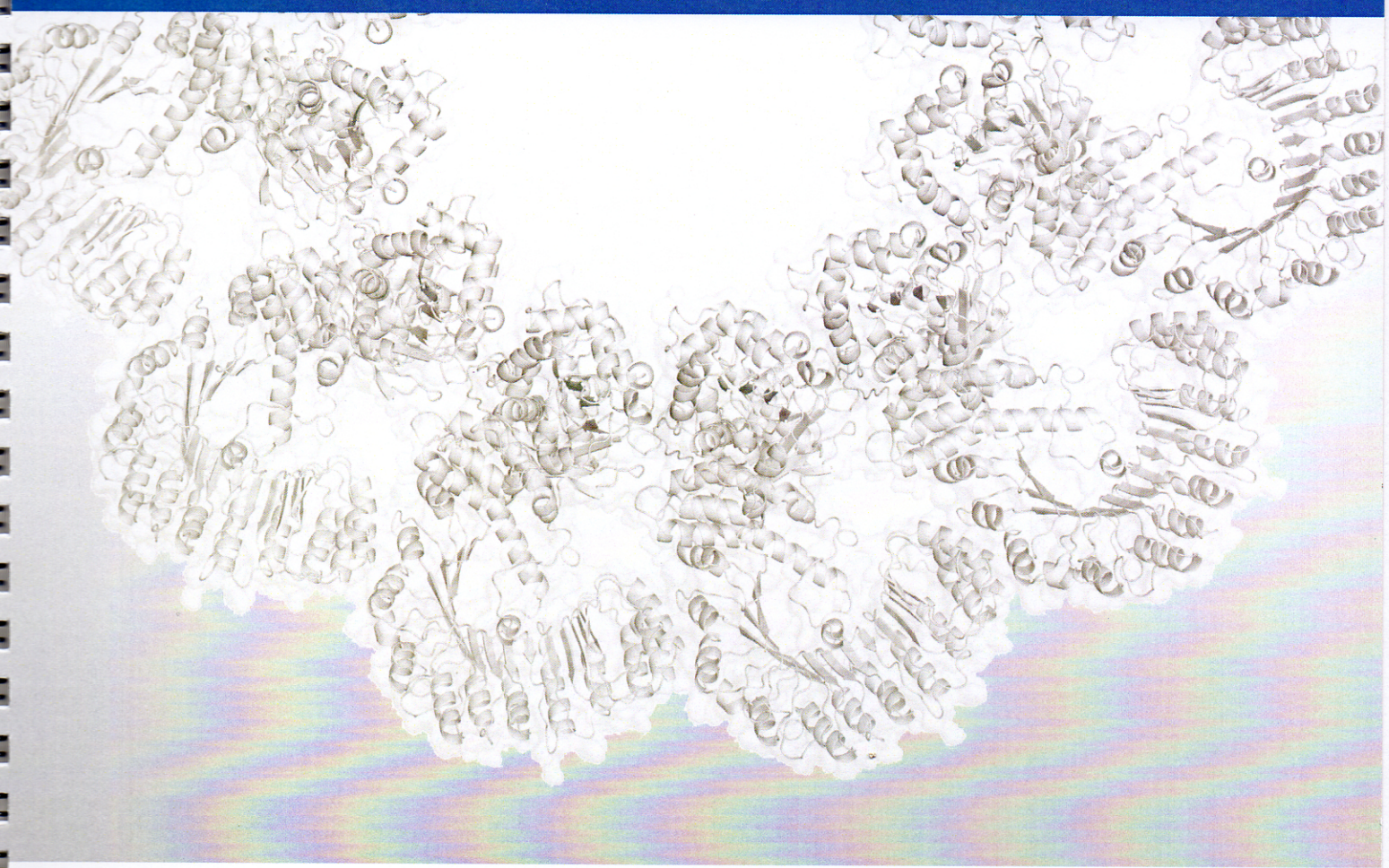


The inflammasomes

25 – 28 September 2018 | Martinsried, Germany

Scientific Organizers:
Veit Hornung | Kate Fitzgerald | Felix Meissner | Luke O'Neill



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058 LEGIONELLA TRIGGERS THE AIM2 INFLAMMASOME THAT ENGAGES ACTIVE UNPROCESSED CASPASE-1 TO INDUCE ACTIVATION OF THE NLRP3 INFLAMMASOME

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Inflammasomes are multimeric protein complexes that initiate inflammatory cascades. Their activation is a hallmark of many infectious or inflammatory diseases. Their composition and activity are specified by proinflammatory stimuli. For example, the NLRP3 inflammasome is activated in response to cell damage and K+ efflux, whereas the AIM2 inflammasome is activated in response to cytosolic DNA. We used Legionella pneumophila, an intracellular bacterial pathogen that activates multiple inflammasome types, to elucidate the molecular mechanisms regulating inflammasome activation during infection. Upon infection, the AIM2 inflammasome engaged caspase-1 to induce pore formation in the membranes of infected cells, which then caused K+ efflux mediated activation of NLRP3. Thus, the AIM2 inflammasome amplifies signals of infection, triggering noncanonical activation of NLRP3. During infection, AIM2 and caspase-11 induced membrane damage, which was sufficient and essential for activating the NLRP3 inflammasome. Our data reveal that different inflammasomes regulate each other's activity to ensure an effective immune response to infection.

059 Bacillus anthracis promotes host lethality through a RIPK1/caspase-8 platform that induces either apoptosis or NLRP3-driven pyroptosis in macrophages

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Bacillus anthracis is a potent biological warfare agent that causes anthrax upon ingestion, inhalation or cutaneous exposure. Early myeloid cell cytotoxicity by B. anthracis lethal toxin (LT) has been identified as a major determinant of host lethality. Contrary to BALB/c macrophages that undergo Nlrp1b inflammasome-mediated pyroptosis, the molecular mechanisms by which this major virulence factor triggers macrophage cell death in C57BL/6 and human macrophages is poorly understood. Here we show that by cleaving MKKs, LT disrupts a protective MKK-P38-MK2 checkpoint that suppresses RIPK1 kinase activity-dependent cell death. Consequently, genetic and pharmacological blockade of RIPK1 kinase activity significantly delayed LT intoxication- and B. anthracis infection-induced macrophage cell death Deletion of caspase-8 (and RIP3) further enhanced cell survival. The RIPK1/Caspase-8 platform elicited both apoptotic and NLRP3 inflammasome-driven pyroptotic cell death of LT intoxicated- and B. anthracis-infected macrophages. Finally, caspase-8/RIP3 KO mice showed significantly prolonged survival relative to littermate controls in a B. anthracis infection model. This work greatly increases understanding of the molecular mechanisms underpinning host-B. anthracis interactions, and uncovers novel potential targets for the treatment of anthrax pathology.

060 Bruton's Tyrosine Kinase (BTK) regulates the NLRP3 inflammasome directly through NLRP3 tyrosine phosphorylation

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The NLRP3 inflammasome is a molecular machinery that forms upon NLRP3 oligomerization, recruitment of the adaptor ASC, and caspase-1 binding and auto-proteolytic activation, and leads to the maturation and release of IL-1 family cytokines and other alarmins. Although its pathophysiological importance for inflammatory processes in humans has widely been recognized, the regulatory mechanisms controlling this vital inflammatory process are poorly understood. We previously identified Bruton's tyrosine kinase (BTK) as a novel and direct regulator of the NLRP3 inflammasome. We here show that BTK and NLRP3 associate upon inflammasome activation in both human and mouse primary cells and that this coincides both with tyrosine phosphorylation and a conformational change of endogenous NLRP3. Consequently, tyrosine phosphorylation was abrogated in BTK-deficient murine macrophages or cells from BTK-deficient human patients. NLRP3 modification was strictly dependent on BTK kinase activity and targetable via clinically available BTK kinase inhibitors. Having confirmed that BTK acts directly on NLRP3, we mapped the site of modification and investigated the molecular consequences of tyrosine phosphorylation. Overall, our data contribute to a better understanding of molecular mechanism of NLRP3 inflammasome activation. Given the clinical availability of FDA-approved BTK inhibitors a BTK-centered targeting approach may pave the way for new treatment strategies in NLRP3 inflammasome-linked inflammation.

061 The Viral Tegument Protein pp65 Impairs Transcriptional Upregulation of IL-1β by Human Cytomegalovirus through Inhibition of NF-kB Activity

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Interleukin-1β (IL-1β) is a key effector of the inflammasome complex in response to pathogens and danger signals. Although it is well known that assembly of the inflammasome triggers proteolytic cleavage of the biologically inactive precursor pro-IL-1β into its mature secreted form, the mechanism by which human cytomegalovirus (HCMV) regulates IL-1β production via the inflammasome is still poorly understood. Here, we show that infection of human foreskin fibroblasts (HFFs) with a mutant HCMV lacking the tegument protein pp65 (v65Stop) results in higher expression levels of mature IL-1β compared to its wild-type counterpart, suggesting that pp65 mediates HCMV immune evasion through downmodulation of IL-1β. Furthermore, we show that enhanced IL-1β production by the v65Stop mutant is due in part to induction of DNA binding and transcriptional activity of NF-κB. Lastly, we demonstrate that HCMV infection of HFFs triggers a non-canonical IL-1β activation pathway where caspase-8 promotes IL-1β maturation independently of caspase-1. Altogether, our findings provide novel mechanistic insights into the interplay between HCMV and the inflammasome system and raise the possibility of targeting pp65 to treat HCMV infection.