NEMO meeting report, July 6th 2006, University of Geneva

Members of the NEMO network (Non-mammalian experimental models for the study of bacterial infections), invited speakers, and an audience totaling more than 20 attendees gathered at the University of Geneva for a one day meeting on July 6th, 2006. The aim of the meeting, organized by Pierre Cosson, was to obtain an overview of different non-mammalian systems used to study bacterial pathogens and to communicate recent results obtained with these model systems. Host cells and organisms studied by some of the groups included *Dictyostelium*, *Acanthamoeba*, *Drosophila*, as well as Zebra fish. These hosts were employed to analyze interactions with the bacterial pathogens *Klebsiella*, *Pseudomonas*, *Aeromonas*, *Yersinia*, *Parachlamydia*, *Legionella*, and *Mycobacterium*.

Pierre Cosson (Pierre.Cosson@medecine.unige.ch) opened the meeting with a presentation entitled "Bacterial killing by *Dictyostelium* amoebae". His group is interested in identifying *Dictyostelium* genes required for killing of *K. pneumoniae*, as well as bacterial genes determining survival within host cells. Recently, the *Dictyostelium* genes *PHG1* and *KIL1* were identified and shown to be involved in intracellular killing of *K. pneumoniae* (Benghezal *et al.*, 2006). Phg1 is a member of the 9 transmembrane family of proteins, and Kil1 is a sulphotransferase.

Marie-Odile Fauvarque (marie-odile.fauvarque@cea.fr) introduced to the audience the fruit fly *Drosophila* as a model for innate immunity against bacterial pathogens (Avet-Rochex et al., 2005). In her talk entitled "The protein phg1A/TM9sf4 contributes to hemocyte-dependent phagocytosis and innate immunity in *Drosophila*" M.-O. Fauvarque focused on the role of the Phg1 protein in phagocytosis and the induction of antimicrobial peptides by the Toll receptor-dependent pathway. Interestingly, similar to *Dictyostelium*, *Drosophila* mutants lacking PHG1 also exhibited a specific susceptibility to *K. pneumoniae* infections (Benghezal *et al.*, 2006).

Johanna Chluba (johanna.chluba@u-bourgogne.fr) studies innate immunity and especially the Toll-like (TLR) family of receptors in Zebra fish (*Dania rerio*) (Jault *et al.*, 2004). Her presentation was entitled "Zebrafish as an animal model in biomedical research" and gave an overview on genetic techniques available for Zebra fish to study host pathogen interactions. These techniques include the microinjection of fluorescent morpholino analogues to knock-down genes of interest, the construction of transgenic fish, as well as the production of xenografts consisting of human cells.

Juan Tomas (jtomas@ub.edu) works on bacteria of the genus *Aeromonas*, including fish pathogens. His talk "Some pathogenic features of mesophilic *Aeromonas*" summarized current knowledge on the classification and virulence traits of *Aeromonas* spp. These bacteria produce exotoxins (hemolysin, phospholipases), a type III secretion system, as well as polar and lateral flagella, which are involved in pathogenesis. Notably, an *A. hydrophila* strain lacking the type III secretion system shows reduced cytotoxicity for fish macrophages and is impaired for virulence (Vilches *et al.*, 2004).

Barbara Weissenmayer (bweiss@zedat.fu-berlin.de) delivered a talk on "*Dictyostelium discoideum* as a functional model organism for the study of *Yersinia* virulence factors". In her presentation, she characterized the subcellular targeting and physiological effects of the *Yersinia* effector proteins YopE (a GTPase activating protein) and YopM (an effector of unknown function), upon heterologous expression in *Dictyostelium*. While YopE-GFP was found to localize to the Golgi apparatus and to retard growth of *Dictyostelium*, YopM-GFP localized to the nucleus.

Gilbert Greub (Gilbert.Greub@chuv.ch) and his group study the interactions of the obligate intracellular bacteria *Parachlamydia* with environmental amoebae, such as *Acanthamoeba castellanii*, and other phagocytes (macrophages). In his talk, entitled "Recognition of *Parachlamydia* by Toll-like receptors", he outlined that *P. acanthamoebae* interferes with endocytic maturation and resides in an acidic intracellular compartment, which stains positive for markers of late endosomes and lysosomes (LAMP-1, lysotracker) (Greub *et al.*, 2005). Furthermore, uptake of the bacteria by macrophages was found to be independent of TLR 2 and TLR4.

Hans Faix (faix@bpc.mh-hannover.de) is interested in the structure, function and dynamics of the *Dictyostelium* cytoskeleton (Faix & Rottner, 2006). He described a REMI (restriction insertion mutagenesis screen) for host factors required for intracellular replication of *Legionella*. The *Dictyostelium* REMI mutants were infected with GFP-labeled *L. pneumophila*, sorted by flow cytometry for *Dictyostelium* mutants harboring green fluorescent *L. pneumophila*, and the gene insertions in *Dictyostelium* clones surviving the infection with the bacteria were identified by inverse PCR.

Hubert Hilbi (hilbi@micro.biol.ethz.ch) and his group use amoebae, including *Dictyostelium*, and macrophages to study the interactions of *Legionella* and *Shigella* with phagocytic cells. His

presentation was entitled "Subversion of phosphoinositide metabolism by *Legionella*" and described recent data demonstrating that *Dictyostelium* phosphoinositide-3 kinases suppress intracellular growth of *L. pneumophila* (Weber et al, 2006). Moreover, *L. pneumophila* was found to secrete effector proteins into host cells, which bind to specific phosphoinositides on the *Legionella* vacuole. Thus, *Legionella* exploits host cell phosphoinositide metabolism to establish its replicative niche.

Thierry Soldati (Thierry.Soldati@biochem.unige.ch) analyzes the interactions between *Mycobacterium* and *Dictyostelium* by using cell biological and biochemical assays (Gotthard *et al.*, 2006). Monica Hagedorn from the Soldati group spoke about "Early events in the establishment of infection of *Mycobacterium marinum* in *Dictyostelium*". Using a variety of techniques it was shown that the pathogen *Mycobacterium marinum* inhibits phagosomal maturation in *Dictyostelium* and replicates intracellularly leading to host-cell "lysis" within 48 hr of infection. Furthermore, the mycobacterial macrophage-activated-gene 24 (mag24-2) was found to be activated upon intracellular pathogen proliferation.

Overall, the meeting was very successful in bringing together scientists interested in pathogenic bacteria and non-mammalian hosts. During and after the oral presentations, lively discussions among the speakers and the audience took place. The attendees of the meeting agreed that this stimulating event should be repeated in the near future.

References

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Hubert Hilbi, August 2006.