NEMO 2006

NON-MAMMALIAN **E**XPERIMENTAL **MO**DELS
FOR THE STUDY OF BACTERIAL INFECTIONS

Coordinator: Pr. P. Cosson (Pierre.Cosson@medecine.unige.ch)

Progress Report 2006

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To test the ability of a bacteria to cause a disease it is usually necessary to infect a mammalian host and allow the disease to progress. These experiments inflict significant suffering to the animals. Our general aim is to stimulate the emergence of a community of scientists using alternative non-mammalian hosts for the study of bacterial infections. Our common belief is that many experiments currently carried out using mammalian hosts could be advantageously replaced by the use of alternative non-mammalian hosts.

Our network of laboratories was created in Feb 2005, initially as an informal gathering of research groups involved in similar subjects. Our specific goals are:

1-To organize an annual meeting on the theme of Non-mammalian hosts for the study of bacterial infections, in order to stimulate exchanges among research groups.
2-To strengthen our research in this field through a series of collaborative works, for which we hope to find financial support.
3-To publicize the use of alternative non-mammalian hosts in the scientific community.

The five research groups currently implicated in this network are: P. Cosson (Geneva, CH), M.O. Fauvarque (Grenoble, FR), G. Greub (Lausanne, CH), H. Hilbi (Zurich, CH) and T. Soldati (Geneva, CH). We might invite a few other research groups to join us in the future.

In this Progress Report we describe briefly the results obtained in 2006. A summary of the 2006 NEMO meeting is also attached.

ANALYSIS OF BACTERIAL VIRULENCE IN DICTYOSTELIUM

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Our general aim is to show that using the unicellular amoeba Dictyostelium, we can study the mechanisms which render bacteria pathogenic, as well as mechanisms allowing infected hosts to fight invading pathogenic bacteria. This approach has a huge potential to reduce the use of animal experiments, since it is today standard to study bacterial infections in living mammals, usually mice. It should be stressed that the use of an alternative non-mammalian host is not necessarily a limitation to research: in several of our studies, the simplicity of the experiments allows experiments that would simply no be feasible in animal models. Our recent results are briefly summarized below.

1- Host genes that determine resistance to pathogens

We previously reported the identification and characterization of genes that determine resistance of phagocytic cells to various bacterial pathogens. The first results were published recently (Benghezal et al., 2006). Since then we have performed several systematic genetic screens to isolate new host genes that determine resistance to various pathogens. This work is still ongoing, but several essential notions are emerging: 1-it is feasible to isolate new host resistance genes using the strategy that we have defined, 2-many host resistance genes are important for resistance to only a subgroup of pathogenic bacteria. Thus the corresponding mutants are highly susceptible to certain pathogens, but not to others. 3-the first genes isolated, which were analyzed in more detail, play specifically a role in the intracellular killing of internalized bacteria. Indeed the mechanisms that ensure intracellular killing of bacteria by cells of the immune system are poorly characterized, and this could be a new model to tackle this difficult question.

Finally, we have performed detailed studies of the organization and function of the endocytic pathway in Dictyostelium (Cherix et al., 2006; Mercanti et al., 2006; Charette and Cosson, 2006). Although not directly connected to the study of host resistance, we expect that these studies will help us to understand how bacteria are endocytosed and killed by phagocytic cells.

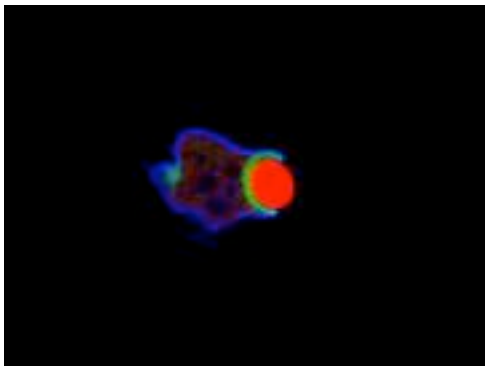

Fig. 1: A Dictyotelium amoeba (blue) internalizing a yeast particle (red). The contact area is labelled in green. This system allows a detailed study of the mechanisms by which a cell internalizes and kills different types of pathogenic bacteria and constitutes an alternative to animal models for the study of bacterial pathogens.

2 - The study of bacterial virulence

With a specific support from the 3R Foundation, we have shown that Dictyostelium constitutes a relevant model to study the pathogenesis of several pathogenic bacteria, in particular Pseudomonas aeruginosa (Manuscript in revision) and Klebsiella pneumoniae (Benghezal et al., 2006).

3- Publications 2006:

*** Support by 3R explicitly mentioned.**

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BACTERIAL VIRULENCE AND INNATE IMMUNE RESPONSE: *DROSOPHILA* AS A MODEL SYSTEM

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1- Evolutive conservation of the innate immune response

Innate immune response is essential for the elimination of pathogens and, in the case of Vertebrates, for the activation of the adaptative immune system allowing the production of specific antibodies. Pathogens recognition and engulfment relies mainly on epithelial cells and phagocytic cells from the blood cell lineage that also synthesise signalling molecules contributing to the production of inflammatory cytokines and of antimicrobial peptides by immune organs. The innate immune signalling pathways include NF-κB, Jun kinases- and p38-MAP Kinases-dependent pathways that are conserved in most live organisms. In particular, genetic and molecular studies have revealed a striking conservation between the mechanisms that regulate insect host defence and the mammalian innate immune response. In *Drosophila melanogaster* flies, activation of Pattern Recognition Receptors induces a humoral response that mainly consists in NF-κB-dependent antimicrobial peptide synthesis by the fat body (the functional equivalent of mammalian liver). Peptide synthesis is stimulated by two distinct signalling pathways, the Toll and the Immune deficiency (Imd) pathways, which are similar to the Toll-like receptor and the Tumour Necrosis Factor receptor signalling pathways in mammals, respectively. In addition, *Drosophila* blood cells of the hemocyte lineage can differentiate into phagocytes ensuring pathogens engulfment.

During infection, pathogens synthesize a vast number of virulence factors targeting host defence mechanisms. In particular, the type III secretion system is a cell-to-cell contact-dependent apparatus that uses a needle-like structure to deliver toxic bacterial proteins either to the cell membrane or directly into the host cell cytoplasm. The type III secretion system is found in many Gram-negative pathogens including *Pseudomonas aeruginosa*, an opportunist pathogen that causes nosocomial infections and is the major cause of death in cystic fibrosis patient. Others and we have shown recently that virulence factors characterized previously in mammalian systems, including quorum-sensing and type III secretion system, also play a role in *P. aeruginosa* virulence against *D. melanogaster* and *D. discoideum*.

2 - Results

Various genetic approaches in *Drosophila* led us to identify new proteins involved in host defence against bacterial infections :

1) We developed a new transgenic approach that allowed us to analyze the contribution of the *P. aeruginosa* exotoxin S to bacterial virulence *in vivo*, and to identify new ExoS targets involved in inflammation and innate immune response (Avet-Rochex et al., 2005).

2) A genetic screen in Drosophila allowed us to identify conserved proteins involved in bacterial resistance. Notably, we selected a deubiquitinylating enzyme whose expression decreases flies resistance to infection. We further demonstrated that this protein is involved in the negative regulation of the Imd/NK-kB-dependent antimicrobial peptide synthesis in response to infection. We are currently identifying its substrates and partners.

3) We continued our collaboration with the laboratory of Pr. P. Cosson in order to analyse the interaction of pathogenic bacteria with non-mammalian hosts, respectively *Dictyostelium* amoebae and *Drosophila* flies.

- We have demonstrated that *Pseudomonas* mutants exhibiting decreased virulence in a *Dictyostelium* system also show decreased virulence in *Drosophila*. The mode of infection is crucial to determine the role of a given gene product: all mutants analyzed were still pathogenic following septic injury into the thorax or induced only slightly delayed mortality, while when fed to the flies, mutant bacteria lost their pathogenicity compared to wild type controls. This suggests that the virulence genes identified in this study play a crucial role in the early stages of a natural infection (such as the passage of the intestinal epithelium), but are dispensable at later stages (Alibaud et al., in revision).

- Dictyostelium mutants with increased sensitivity to certain pathogenic bacteria (in particular *Klebsiella pneumoniae* and *Pseudomonas aeruginosa*) were obtained and characterized in the laboratory of Pierre Cosson including a gene called PHG1. A mutant *Dictyostelium* strain defective for PHG1 exhibited a specific susceptibility to *Klebsiella pneumoniae* bacteria due to its essential role in the intracellular killing of *K. pneumoniae* bacteria (Benghezal et al. 2006). *PHG1* encodes a polytopic membrane protein with a N-terminal lumenal domain and nine potential transmembrane segments. The existence of *phg1* orthologs in mammals and *Drosophila* (*Dphg1*) suggests that it may play similar function in these organisms. To assess this question, a *Drosophila* strain where *Dphg1* is deleted was created in our laboratory and its function in *Drosophila* immunity has been characterized (Bergeret et al., submitted). Remarkably we observed that Drosophila *phg1* mutants exhibited increased sensitivity to infection with *K. pneumoniae* (Benghezal et al., 2006). This result illustrates the advantage of our combined approach of bacterial pathogenesis. We are currently creating mutation on the two others *Phg* genes in drosophila in order to get an integrated view of this new family of proteins in innate immunity and development.

3- Conclusion:

The general aim of the team is to identify new mechanisms of innate immunity by original approaches in *Drosophila* and to elucidate the contribution of bacterial virulence factors in inhibiting host defence mechanisms. We will also analyze molecular and cellular dysfunction caused by a deregulation of these candidate genes *in vivo* during development and adult life.

4- References:

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Free-living amoebae as a tool to test the bacterial virulence

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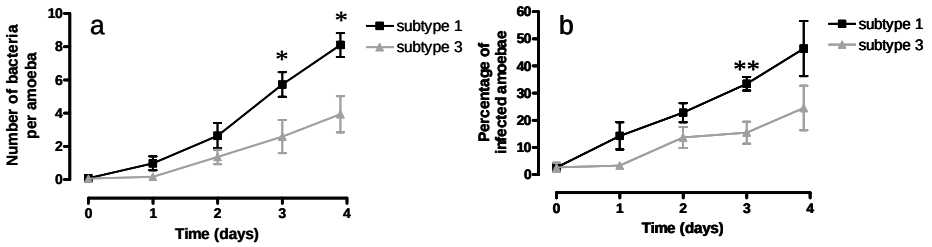
Free-living amoebae feed on bacteria, fungi and algae. However, some microorganisms evolved to become resistant to these protists. These amoebae-resistant microorganisms include established pathogens, such as *Legionella*, *Mycobacterium avium*, and *Listeria monocytogenes*, and emerging pathogens, such as *Parachlamydia acanthamoebae* (reviewed in (1;2)). Thus, free-living amoebae may be used as a tool for the culture of some intracellular bacteria and new bacterial species that might be potential emerging pathogens (2;3). We therefore used amoebae as cells in a cell culture system to isolate novel hosts of free-living amoebae from water. The potential role played by these intracellular bacteria as agents of pneumonia is then studied (i) since human are commonly exposed to water and (ii) since a bacteria that resists to amoebae is likely able to resist also to the microbicidal effectors of alveolar macrophages, i.e. the main innate immune cells in the lung (2).

During last years, the research done by G. Greub *et al.* mainly focused on Chlamydia-like organisms, *Mycobacteria* and *Legionella pneumophila*. Using the amoebal co-culture and amoebal enrichment approach, we isolated a large number of new amoebae-resisting micro-organisms from the water of Lausanne's University hospital (4) and from the Seine river (5). In order to select highly virulent bacteria that will be further studied for their role in pneumonia, we had to develop a screening approach to test each new recovered species. Thus, we investigated during the last year whether free-living amoebae might be used as a tool to determine the virulence of a given bacterial species. We studied virulent and avirulent *Mycobacteria* [*M. kansasii*] (6) for their ability to grow within the *Acanthamoeba* amoeba.

Mycobacterium kansasii is (with *Mycobacterium avium*), one of the more common mycobacterial species responsible for disease due to nontuberculous mycobacteria (6). However, little is known about its pathogenicity, mode of transmission, and natural reservoir. *M. kansasii* causes pulmonary disease similar to tuberculosis in immunocompetent patients and pulmonary, extrapulmonary, or disseminated disease in patients with various immunodeficiencies, in particular HIV infection. We showed previously that subtypes 1 and 2 are more pathogenic than subtype 3 (6). Consequently, in this project, we tested whether the growth of *Mycobacterium kansasii* in *Acanthamoeba* correlates with the virulence of *M. kansasii*.

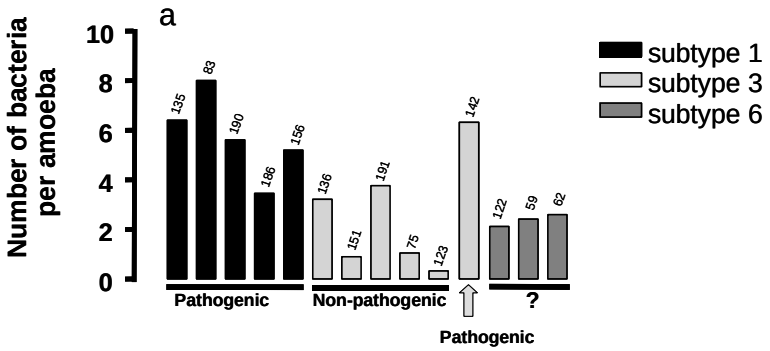
Pathogenic subtype 1 *M. kansasii* strains grew better in *A. castellanii* than non-pathogenic subtype 3 strains, when considering both the number of bacteria per amoeba and the percentage of infected amoebae (Figure 1).

Figure 1. Mean number of bacteria per amoeba (a) and percentage of infected amoebae (b) following infection of *A. castellanii* ATCC 30010 with *M. kansasii* subtype 1 strains (n=5) and subtype 3 strains (n=5). Error bars show the standard error of the mean. *: p-value <0.05; **: p-value <0.01.



Moreover, a subtype 3 *M. kansasii* strain isolated from blood culture, and thus considered pathogenic, revealed to grow in *A. castellanii* similarly to pathogenic subtype 1 strains (Figure 2).

Figure 2. Mean number of bacteria per amoeba 3 days p.i. with each *M. kansasii* strains studied [pathogenic subtype 1 (n=5), for non-pathogenic subtype 3 (n=5), for pathogenic subtype 3 (strain n°142) and for subtype 6 (n=3)]. The number indicated above each bar is the strain number. (b) Mean number of bacteria per amoeba from day 0 to day 4 for. The data shown are the mean of 3 replicates.



These results suggest that amoebae may represent a useful tool to test the virulence of intracellular mycobacteria and of other amoeba-resisting bacteria. Since this system based on amoebal co-culture is amenable to large screenings, in 24 or 96-wells plates, it may potentially be used to screen mutants for virulence factors. Although the *Acanthamoeba* amoeba has been less well characterized genetically than *Dictyostelium discoideum*, *Acanthamoeba* has the advantage over *Dictyostelium* to remain viable and to grow at temperatures above 25 °C. Thirty-two to 37°C represent the temperature range at which bacteria encounter human macrophages during the process of infection and the *Acanthamoeba* model may thus more accurately reflect the *in vivo* setting of human infection. Moreover, since animal models are generally used to test bacterial virulence, this alternative non-mammalian experimental model we developed to test the virulence of intracellular bacteria may also reduce the use of animal. This work is currently in press in “Research in Microbiology” (7).

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AMOEBAE: A CELLULAR PATHOGENESIS MODEL FOR THE LEGIONNAIRES' DISEASE AGENT *LEGIONELLA PNEUMOPHILA*

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BACKGROUND

Legionella pneumophila is an accidental bacterial pathogen, which may cause the severe pneumonia Legionnaires' disease. In the environment *L. pneumophila* colonizes biofilms and replicates within amoebae including the social amoeba *Dictyostelium discoideum*. Intracellular replication takes place in a specific vacuole, which is formed by means of the bacterial Icm/Dot type IV secretion system (T4SS) and more than 30 secreted "effector" proteins (Fig. 1A). For most of these effector proteins it is not known how they contribute to the subversion of host cell vesicle trafficking and formation of *Legionella*-containing vacuoles (LCV). We currently focus on the Icm/Dot-secreted protein SidC and its paralogue SdcA, which bind to the cytoplasmic side of LCV.

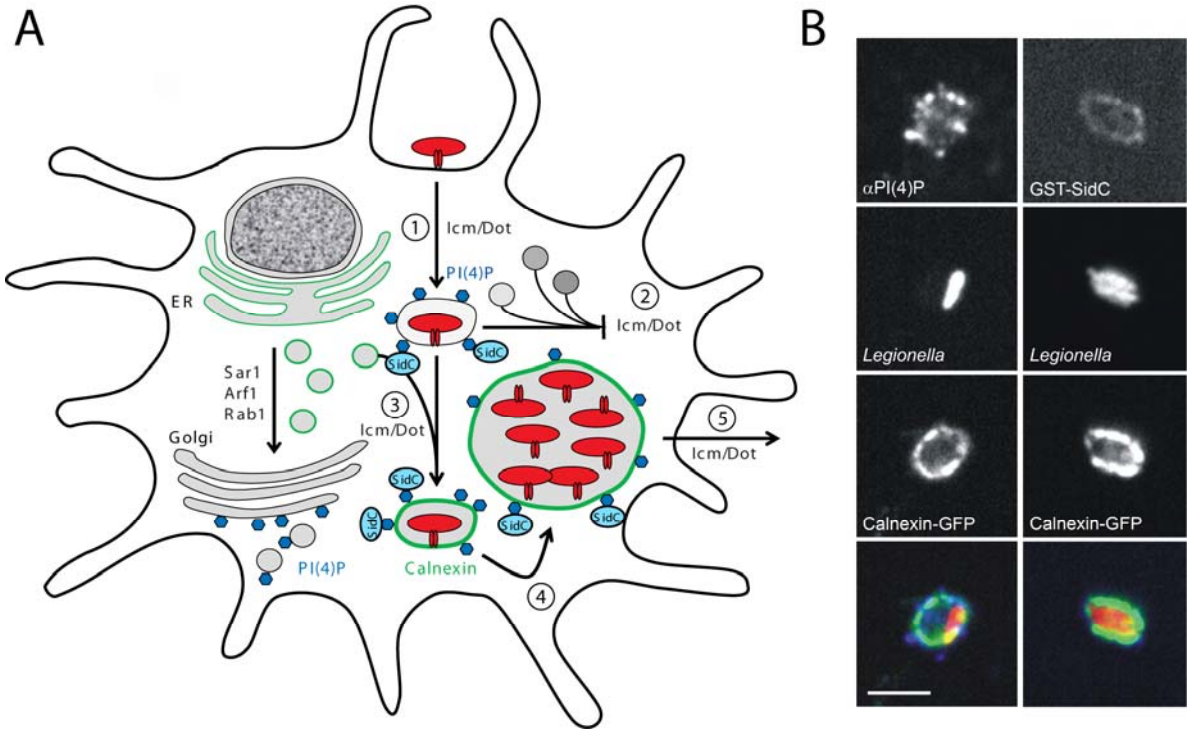

Fig. 1. *L. pneumophila* replicates within a specific vacuole in amoebae. (**A**) The Icm/Dot T4SS of *L. pneumophila* governs ① phagocytosis, ② inhibition of endocytosis, ③ interaction with the early secretory pathway and ⑤ release from amoebae. *Legionella*-containing vacuoles (LCV) accumulate phosphatidylinositol-4 phosphate (PI(4)P), which is bound by the Icm/Dot-secreted effector protein SidC, and acquire ER markers such as calnexin. Formation of the LCV depends on the activity of the small host cell GTPases Sar1, Arf1 and Rab1 as well as on Icm/Dot-secreted bacterial guanine nucleotide exchange factors. ④ Replication of *L. pneumophila* in LCV does not seem to require the Icm/Dot T4SS. (**B**) Isolated LCV from calnexin-GFP expressing *D. discoideum* infected with DsRed-expressing *L. pneumophila* were labeled for PI(4)P with an anti-PI(4)P antibody (left panels) or with purified GST-SidC fusion protein and an anti GST antibody (right panels). Scale bar, 2 μm. Modified after Weber *et al*., 2006a.

RESULTS

***Legionella pneumophila* exploits phosphatidylinositol-4 phosphate to anchor secreted effector proteins to the replicative vacuole** (Weber *et al.*, 2006a).

An analysis of the role of host cell phosphoinositide (PI) metabolism during uptake and intracellular replication of *L. pneumophila* revealed that class I PI(3) kinases (PI3K) are dispensable for phagocytosis of wild-type *L. pneumophila* but inhibit intracellular replication of the bacteria in *Dictyostelium* and participate in the modulation of LCV. Uptake and degradation of an *icmT* mutant strain lacking a functional Icm/Dot T4SS was promoted by PI3K. The Icm/Dot-secreted protein SidC specifically binds to PI(4)P *in vitro* and preferentially localizes to LCV in the absence of functional PI3K. PI(4)P was found to be present on LCV using as a probe either an antibody against PI(4)P, the PH domain of the PI(4)P-binding protein FAPP1 (phosphoinositide 4-phosphate adaptor protein-1) fused to GST, or purified GST-SidC fusion protein (Fig. 1B). Moreover, the presence of PI(4)P on LCV required a functional Icm/Dot T4SS. These results indicate that *L. pneumophila* modulates host cell PI metabolism and exploits the Golgi lipid second messenger PI(4)P to anchor secreted effector proteins to LCV.

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THE AMOEBA *DICTYOSTELIUM DISCOIDEUM* AS A MODEL HOST TO STUDY *MYCOBACTERIUM MARINUM* INFECTION

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1. Background: establishing *Dictyostelium* as a host model to study mycobacteria infection.

The genetically tractable multicellular organisms such as *Drosophila* and zebrafish are instrumental to gain access to the role of innate and acquired immunity. However, because of the universality of virulence mechanisms, single cell systems such as *Dictyostelium* offer a unique chance to investigate fundamental aspects of evolutionarily conserved intracellular immunity in a simplified background. Molecular, cellular, genetic and proteomic studies of the intracellular trafficking pathways have revealed a high degree of conservation with mammalian and other animal models (Hagedorn et al, 2006; Gotthardt et al, 2006a and b). Recently, it has been described as a useful model to study conserved host-pathogen interactions with *Pseudomonas*, *Legionella* and *Vibrio cholera*. Two pioneering studies have indicated the feasibility to use *Dictyostelium* as a model host for mycobacteria.

Pathogenic mycobacteria, among which the causative agent for tuberculosis *Mycobacterium tuberculosis*, pose a major threat to public health. Generally, pathogenic mycobacteria initiate infection by entering the host macrophages via the normal phagocytosis process but then rapidly block the biogenesis of a bactericidal phagolysosome. This leads to the generation of a friendly environment that allows intravacuolar multiplication. *Mycobacterium marinum*, a close relative to *M. tuberculosis*, is a fish and amphibian pathogen that causes systemic granulomatous diseases in its natural hosts. The course of infection, granuloma formation and structure are very similar to the infection and lesions caused by *M. tuberculosis*. It has thus become an established model to study the pathogenesis of tuberculosis in alternative genetically tractable host models such as zebrafish and *Drosophila*.

2. Characterisation of an initial unitary cycle of infection

In the last two years, we established the amoeba *Dictyostelium* as a model host to study mechanisms of infection by *Mycobacterium marinum* (Hagedorn and Soldati, submitted). It was our goal to dissect the balance of host-pathogen interactions during the early phases of infection at a high temporal resolution. In our experimental conditions, *Dictyostelium* provided an efficient host for intracellular *M. marinum* proliferation (Fig. 1). Up to 43 hpi, the changes in CFUs followed a distinct temporal pattern of three phases. The number of CFUs did not increase during an initial phase lasting until 12 hpi (Fig 1a, phase 1), but between 12 and 37 hpi a major proliferation phase was observed, with a 2 to 6 fold increase in the number of CFUs per ml of infected culture (phase 2) peaking at 37 hpi and followed by a plateau or relative decrease (phase 3). The non-pathogenic strain *M. smegmatis* and the avirulent *M. marinum* mutant strain L1D showed no overall increase of CFUs, however, they significantly differed in the dynamics of decline in CFUs. Initially, the number of CFUs for *M. smegmatis* dropped at least 100 fold within the first 12 hpi (Fig. 1 inset), followed by a steady but milder decrease. In contrast, *M. marinum* L1D CFUs followed a gentle but constant decrease (10 to 100 fold within 43 hpi). We conclude that the latter relatively flat profile likely reflects a balance between (low level of) proliferation and (inefficient) killing.

In summary, even though *M. smegmatis* is rapidly killed by *Dictyostelium*, the delayed pattern of events indicates that the endocytic pathway is heavily manipulated by this non-pathogenic bacterium. *M. marinum L1D* is avirulent and exocytosed already after 1 hpi. This is a rate similar to the one determined for an inert particle, a latex bead or common bacteria such as *E. coli* and *Klebsiella*. Together the data indicate that very early presence of the gene product MAG24-1 (absent in the L1D mutant) is essential for pathogenic *M. marinum* to establish an infection. The data also emphasise that during the course of infection, the profiles

observed result from a dynamic balance between bacteria proliferation and killing by the host. Changes can thus be due to inhibition/promotion of proliferation in place of or in addition to increased/decreased killing efficiency.

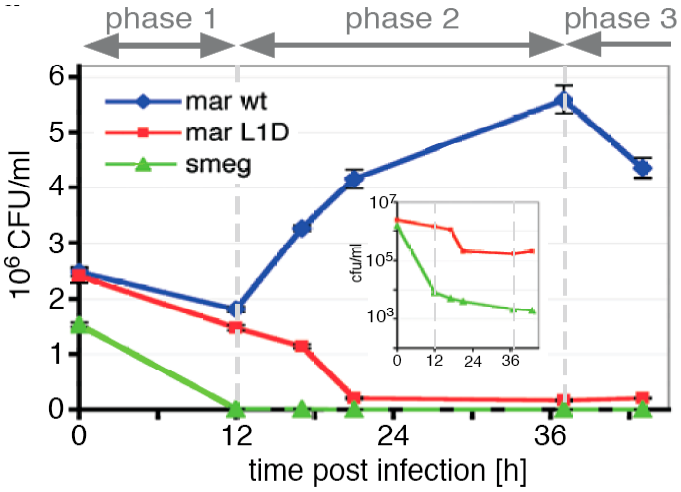

Figure 1. Characterisation of a unitary cycle of infection, proliferation and release. Uptake of wild type *M. marinum* (mar wt), avirulent *M. marinum* L1D (mar L1D) and non-pathogenic *M. smegmatis* (smeg) was synchronised by centrifugation onto adherent *Dictyostelium* cells. After removal of uningested mycobacteria, incubation was performed from 0 hpi up to 43 hpi. At the indicated times, the concentration of live bacteria in the infection mix was determined by CFU counting. The error bars indicate ± SEM of experiments performed in triplicates

Early manipulation of the host phagocytic pathway during phase 1

In order to determine if, when and to which extent *M. marinum* induces a bifurcation from the normal phagosomal maturation route in *Dictyostelium*, the extent of colocalization of bacteria containing phagosomes with well-defined maturation markers {Gotthardt, 2006 #1500; Gotthardt, 2002 #1458} was determined by immunofluorescence. Our results indicated that phagosomes containing *M. marinum L1D* and *M. smegmatis* follow the normal sequence of maturation albeit at very different kinetics. The non-pathogenic *M. smegmatis* nevertheless seems to manipulate phagosomal maturation by slowing it down. In contrast, the majority of phagocytosed *M. marinum* successfully induce a bifurcation in the maturation pathway and thus escape accumulation of the proteins that mark the genesis of a bactericidal compartment.

The flotillin homologue vacuolin accumulates at the replication niche during phase 2

The genesis of the replication niche was examined during phase 2. Surprisingly, even though no significant colocalization with the H^+-ATPase and cathepsin D was observed during phase 1, we detected vacuolin around *M. marinum* at the beginning of phase 2. Most strikingly, at the end of phase 2, *M. marinum* was often present in large apparently spacious vacuolin-positive compartments (Fig. 2).

The gradual and strong accumulation of vacuolin around *M. marinum* during phase 2 allowed us to follow the morphological changes that the replication niche underwent concomitantly with intravacuolar bacteria proliferation. The replication niche started as a tight compartment around the bacteria but, following the growth of bacteria, it became distended and apparently spacious (stage 1). Accompanying replication, the size of the vacuole increased and its membrane was more frequently pushed from inside by the growing bacteria rods, producing sharp deformations of the vacuolar membrane (stage 2). At later stages (21 to 37 hpi), distortion of the vacuole eventually led to its rupture and to bacteria release into the cytosol (stage 3). Bacteria were frequently observed associated with apparently stable sheet-like remnants of their ruptured compartments, but were also found in the cytosol of cells exhibiting completely disorganised vacuolin-positive compartments (stage 4). Observation of that complete sequence of events was not limited to staining against

vacuolin. P80, a putative copper transporter ususally present throughout the endocytic and phagocytic pathways was found around *M. marinum* during all phases of infection

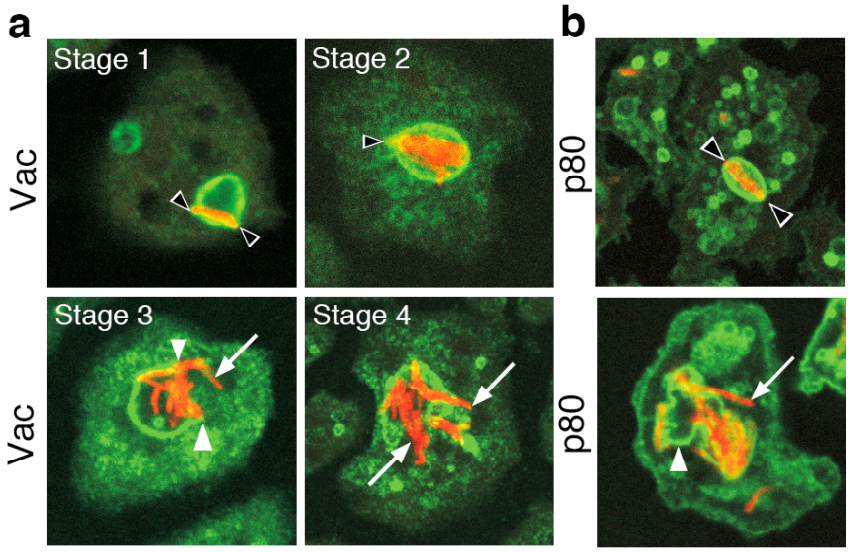

Figure 2. Establishment and rupture of the proliferation niche of *M. marinum* in *Dictyostelium*. Uptake of the three mycobacteria strains was performed as in Fig. 1 and the fate of cells containing fluorescent mycobacteria was monitored by immunofluorescence staining against the phagosome maturation markers cathepsin D (CatD) and vacuolin (Vac). Close inspection of various time points during phase 2 of infection revealed four sequential stages of establishment and rupture of the vacuolin-positive vacuole (a). The putative copper transporter, p80 is also enriched at the delimiting membrane of the proliferation niche, and maybe also on internal membrane profiles. Scale bars 5 μm.

We hypothesise that the unexpected long half-life of remnants of the ruptured vacuole, often in the apparent form of sheets with free edges, might result from stabilisation by the densely packed vacuolin coat. It is also puzzling that, after spending hours establishing and maintaining a safe niche for proliferation, bacteria are released into the cytosol, a biochemically and metabolically completely different environment. It has been proposed that the extRD1 locus of pathogenic mycobacteria is responsible for the secretion of a membranolytic activity that involves ESAT-6, but it is not clear yet whether it impacts on vacuole and/or plasma membrane lysis. Anyway, the presence of pathogenic mycobacteria in the cytosol raises a number of fundamental questions that will be the topic of intense future research. Is it a strategy common to other pathogenic mycobacteria, including *M. tuberculosis*? Does it modulate antigen (cross)-presentation? Does further replication occur in the cytoplasm? Is autophagy involved in controlling pathogenesis? Does escape from the vacuole play a role in dissemination?

3. Publications for the year 2006

Hagedorn, M., and Soldati, T. (submitted) Identification of *Dictyostelium* proteins that modulate intracellular immunity to *Mycobacterium marinum* infection.

Gotthardt, D., Blancheteau, V., Bosserhoff, A., Ruppert, T., Delorenzi, M., and Soldati, T. (2006) Proteomic fingerprinting of phagosome maturation reveals a role for a Gα during uptake. **Mol. Cell. Proteomics.** 5, 2228-2243.

Yoshida, K., and Soldati, T. (2006) Dissection of amoeboid movement into two mechanically distinct modes. **J. Cell Sci.** 119, 3833-3844.

Hagedorn, M., Neuhaus, E.N., Soldati, T. (2006) Optimised fixation and immunofluorescence protocols for *Dictyostelium* cells. **Methods Mol. Biol.** 346, 327-338.

Gotthardt, D., Dieckmann, R., Blancheteau, V., Kistler, C., Reichardt, F., and Soldati, T. (2006) Preparation of intact, highly purified phagosomes from *Dictyostelium* **Methods Mol. Biol.**, 346, 439-448.

Soldati, T., and Schliwa, M. (2006) Powering membrane traffic in endocytosis and recycling. **Nat. Rev. Mol. Cell Biol.** 7, 897-908.

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.

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Hubert Hilbi, August 2006.