Microreview

Dictyostelium as host model for pathogenesis

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Summary

The haploid social soil amoeba Dictyostelium discoideum has been established as a host model for several pathogens including Pseudomonas aeruginosa, Cryptococcus neoformans, Mycobacterium spp. and Legionella pneumophila. The research areas presently pursued include (i) the use of Dictyostelium wild-type cells as screening system for virulence of extracellular and intracellular pathogens and their corresponding mutants, (ii) the use of Dictyostelium mutant cells to identify genetic host determinants of susceptibility and resistance to infection and (iii) the use of reporter systems in Dictyostelium cells which allow the dissection of the complex host-pathogen cross-talk. The body of information presented in this review demonstrates that the availability of host cell markers, the knowledge of cell signalling pathways, the completion of the genome sequencing project and the tractability for genetic studies qualifies Dictyostelium as the study of fundamental cellular processes of pathogenesis.

Introduction

Whether or not host organisms become infected by pathogens is the result of a complex interplay between host and pathogen genotypes, as well as the physiological condition of both species. Nonetheless, most of the previous work in pathogenesis research has focused on the pathogen side of infection. More recently, the host susceptibility to infection receives increasing attention. New approaches in this respect were catalysed by the observation that so-called model organisms like Dictyostelium discoideum, Caenorhabditis elegans and Drosophila melanogaster can be infected by human pathogens (Tan et al., 1999; Hägele et al., 2000; Tzou et al., 2002; Steinert et al., 2003). The main reason for the development of such infection models is the possibility to systematically analyse infection-relevant host cell factors on a molecular level. In this review, we will focus on the interaction of the social soil amoeba D. discoideum with four environmental pathogens, Pseudomonas aeruginosa, Cryptococcus neoformans, Mycobacterium spp. and Legionella pneumophila. These pathogens have in common that their virulence for mammalian hosts is maintained in the environment. Experimental data indicate that virulence of a number of microbes increases after coculture with protozoa (Brown and Barker, 1999). Over evolutionary time the protozoa–microbe interactions may have generated a pool of virulence traits which preadapted some microbial species as human pathogens (Steinert et al., 2002a; Greub and Raoult, 2004). As protozoa seem to be evolutionary incubators of virulence, it appears justified to use D. discoideum as host model for the analysis of cellular aspects of pathogenesis.

Dictyostelium discoideum is a cooperative, genetically tractable phagocyte

The soil amoeba D. discoideum can internalize a remarkably wide range of particles, including latex beads, yeast, benign bacteria and different pathogenic bacteria (Cardelli, 2001; Maniak, 2003; Steinert et al., 2003). The cells can be grown easily in the laboratory at 24.5°C. As long as nutrients are available, the free-living ameboid cells grow and multiply by binary fission. Upon starvation Dictyostelium cells exhibit an impressive multicellular cooperativity. The solitary cells aggregate by chemotaxis in response to relayed cAMP signals. The aggregate of approximately 100 000 cells undergoes a series of morphogenetic changes (Eichinger and Noegel, 2003). After the formation of a motile slug the differentiation culminates in the formation of a macrocyt in which a zygote forms (Raper, 1984). The genome repertoire of Dictyostelium allows the expression of features like cell-type determination, spatial patterning, altruistic cell death and other fundamentals that are essential in multicellular developing organisms (Eichinger and Noegel, 2003; Ennis et al., 2003). The
almost completed genome sequence of *D. discoideum* (http://dictybase.org) will undoubtedly accelerate these fields of research. The genome size of *Dictyostelium* is between 34 Mb and 40 Mb. The six chromosomes are expected to carry 11 000 genes (Glöckner et al., 2002; Eichinger and Noegel, 2003). Introns are present in most genes and splice junctions known from higher organisms are conserved. 

The genome of *Dictyostelium* will undoubtedly accelerate these fields of research. Although the evolutionary position is located before the branching of metazoa and fungi (Baldauf et al., 2000), all of the cytoskeletal elements of *Dictyostelium* are also found in mammalian cells. Moreover, *Dictyostelium* shares its chemotactic capacity with leukocytes and the process of particle uptake in *Dictyostelium* looks remarkably similar to macrophage phagocytosis (Noegel and Schleicher, 2000; Ruppersberg and Cardelli, 2001). The similarities between the *Dictyostelium* and mammalian cells also extend to membrane trafficking, endocytic transit and sorting events (Solomon and Isberg, 2000; Cardelli, 2001). These intrinsic features of *Dictyostelium* combined with a set of well established molecular tools have already made important contributions to the field of cellular microbiology. In the following, we will describe the application of *Dictyostelium* wild-type (Table 1) and mutant cells (Table 2) as effective screening systems for bacterial virulence as well as cellular models for the analysis of the host side of infection.

### Table 1. Virulence factors of pathogens analysed in the *D. discoideum* host model.

| Species | Virulence trait of pathogen | Effect on infection | References |
|---------|-----------------------------|---------------------|------------|
| *P. aeruginosa* | Quorum sensing system (las, rhl) | Inhibition of host cell growth | Pukatzki et al. (2002); Cosson et al. (2002) |
| | Type III secretion (PscJ) | Host cell lysis | Pukatzki et al. (2002) |
| | Rhamnolipids | Host cell lysis | Cosson et al. (2002) |
| | Antibiotic resistance efflux pump (MexEF-OprN) | Better survival of host cell (Overproduction reduces secretion of other virulence factors) | Cosson et al. (2002) |
| | Cytotoxin (ExoU) | Host cell killing | Pukatzki et al. (2002) |
| *M. avium* | Intracellular growth | Bacteria uptake into host cell | Steenbergen et al. (2003) |
| *M. marinum* | Glycine-rich PE-PGRS family protein (Mag24-1) | Uptake into host cell | Solomon et al. (2000) |
| *C. neoformans* | Capsule | Uptake into host cell | Solomon et al. (2000) |
| *L. pneumophila* | Type IV secretion system (Dot/Icm) | Uptake into host cell | Heuner et al. (2002) |
| | Alternative σ^54 factor (FliA) | Intracellular growth | Steiner et al. (2002b) |
| | Flagellin (FlaA) | Intracellular growth | Steinert et al. (2002b) |
| | Macrophage infectivity potentiator (Mip) | Intracellular growth | Steinert et al. (2002b) |
| | ligA gene | Non-lytic exocytosis | Chen et al. (2004) |
| | Components homologous to SNARE system (LepA, LepB) | Better survival of host cell (Overproduction reduces secretion of other virulence factors) | Chen et al. (2004) |

### Dictyostelium wild-type cells as screening system for *Pseudomonas* mutants

The extracellular pathogen *Pseudomonas aeruginosa* is a ubiquitous environmental bacterium (Goodman and Lory, 2004). It represents a common cause of infections in burn victims and cystic fibrosis patients. Moreover, the nosocomial pathogen can initiate an infection in almost any tissue if the host defence is compromised. Consistent with this phenomenon the host spectrum of *Pseudomonas* is remarkably wide and includes plants, insects and nematodes. This suggests the presence of functionally universal virulence factors or a combination of physiological traits that relate to pathogenesis. The recent completion of the *Pseudomonas* Genome Project (http://www.pseudomonas.com) revealed that one-third of the potential proteins have no sequence similarity to other proteins. As *Pseudomonas* is known to contain unique metabolic pathways and novel secretory mechanisms, we obviously need new tools to understand the functional context of the gene products that lead to antibiotic resistance, cell–cell signalling and pathogen–host interactions.

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By using simple plating assays it was shown that *Pseudomonas* utilizes conserved virulence pathways to infect *Dictyostelium* (Pukatzki et al. 2002). When the amoebae were plated on nutrient agar plates with different *P. aeruginosa* wild-type and mutant strains, the bacteria form lawns on these plates with amoebae embedded in them. Virulent bacterial strains were observed to kill *Dictyostelium* which resulted in an intact bacterial lawn. Certain avirulent mutant strains, however, were eliminated by the amoebae which resulted in plaques within the bacterial lawn (Table 1). The analysed avirulent *Pseudomonas* strains were mutated in a transcription factor relevant for quorum sensing-mediated virulence (LasR), a structural component of type III secretion (PscJ), and the cytotoxin ExoU. The complementation of the *lasR* and *exoU* mutations restored the virulence of the *Pseudomonas* mutants towards *Dictyostelium*.

Another plating assay utilized *Klebsiella pneumoniae* as a marker of phagocytosis (Cosson et al., 2002). This assay revealed that the virulent *P. aeruginosa* strain PAO1 inhibited the growth of *Dictyostelium* on a *Klebsiella* lawn. The use of *Pseudomonas* mutants in this assay demonstrated that factors controlled by the rhl quorum sensing system were necessary for the observed effect. In particular, rhamnolipids were shown to induce lysis of the *Dictyostelium* cells. The analysis of a multidrug-resistant overproducer of the MexEF-OprN efflux system which showed reduced production of elastase, pyocyanin and rhamnolipids was less inhibitory to *Dictyostelium* than the isogenic wild-type strain. This appears to be of general relevance since the finding that the acquisition of antibiotic resistance affects bacterial virulence is not limited to *P. aeruginosa* (Mizobuchi et al., 1994). Interestingly, the authors were able to confirm the validity of this simple *Dictyostelium* plating assay by using the time-consuming and expensive rat model of acute pneumonia (Cosson et al., 2002). Although some conflicting data with regard to the relevance of rhamnolipids and the las quorum sensing system have appeared with the different plating assays, the obtained results confirmed the usefulness of *Dictyostelium* wild-type cells as a screening model for bacterial virulence (Cosson et al., 2002; Pukatzki et al. 2002). In the future, *Dictyostelium* mutant cells may help to identify specific target structures of the opportunist pathogen *P. aeruginosa*.

### D. discoideum mutant cells and the analysis of opportunistic *Mycobacterium* species and the fungus *Cryptococcus neoformans*

Opportunistic infections are seen in patients with impaired host defences such as AIDS, trauma and following immunosuppressive therapy. Classic examples of such diseases include environmental *Mycobacterium* infections and cryptococcal meningitis (Steinert et al., 1998; Collazos, 2003). Both pathogens are found in the environment and upon infection grow intracellularly within human macrophages.

Invasion assays demonstrated that *Mycobacterium avium* is able to survive within *Dictyostelium* cells (Table 1). The intracellular life cycle in *Dictyostelium* vacuoles which results in host cell lysis appears similar to that observed in human macrophages (Skriwan et al., 2002). *Dictyostelium* natural resistance-associated macropage protein (Nramp1)-knockout cells were significantly more permissive hosts than wild-type cells (Table 2). The human homologue of Nramp1 is a divalent cation transporter and polymorphic variants have been associated with an increased susceptibility to tuberculosis and leprosy (B. Peracino et al. unpublished). *M. marinum*, another environmental *Mycobacterium* species, causes
tuberculosis-like diseases in fish, and cutaneous infections in humans. This mycobacterial species also survives intracellularly within *D. discoideum*. A *M. marinum* mutant carrying an insertion in the gene mag24-1, which encodes for a glycine-rich PE-PGRS (polymorphic GC-repetitive sequence) family protein, exhibited reduced growth in *D. discoideum*. The absence of the host cell protein coronin enhanced the yield of intracellular bacteria compared to the wild-type phenotype (Solomon et al., 2003).

*Cryptococcus neoformans* is an encapsulated environmental fungus that infects the human host via the respiratory tract. It usually causes apparent infections; however, in immunocompromised patients the fungus may disseminate and produce a life-threatening meningitis. This saprophytic soil organism is a good example for the adaptation to both amoebae and human macrophages. Virulent encapsulated strains survive intracellularly within human macrophages, *Acanthamoeba castellani* and *D. discoideum*, whereas non-virulent non-encapsulated strains do not (Table 1) (Steenbergen et al., 2003). Phagocytosis of *C. neoformans* by *D. discoideum* can be inhibited with capsule-specific antibodies. Passaging of *C. neoformans* through *D. discoideum* significantly increased virulence for mice and the enhancement of virulence was shown to correlate with capsule size and melanization. Interestingly, incubation of non-virulent non-encapsulated *C. neoformans* with *D. discoideum* mutants defective in myosin VII synthesis resulted in proliferation of the fungus and death of the host cell (Table 2). Similar effects were observed in experiments using *Legionella pneumophila* in which a myosin I double mutant was more permissive to bacterial growth.

Taken together the observed findings of the Mycobacterium and Cryptococcus infections in Dictyostelium mutants validate the concept that the transfer from a healthy host to a weak host can convert an avirulent organism into an opportunistic pathogen (Hentschel et al., 2000). Consequently, the next steps towards the understanding of opportunistic infections will be the identification of exploited host cell structures and structures that normally protect the host. As the Dictyostelium-infection model allows to specifically weaken the host by genetic manipulation, a future strategy may be to test a battery of such Dictyostelium mutants in a high-throughput approach. Dictyostelium LvsB (large volume sphere) mutants which model the lysosomal defects associated with Chediak–Higashi syndrome or Nramp1 mutants and variants (see below) may be good candidates in this respect (Harris et al., 2002).

**Custom-tailored Dictyostelium cells contribute to the roadmap of Legionella infection**

The most advanced application of Dictyostelium as a host model occurred with *L. pneumophila*. Ubiquitously present in aquatic habitats, *L. pneumophila* replicates intracellularly within different species of free-living protozoa. The inhalation of aerosolized legionellae by humans can result in a severe atypical pneumonia called Legionnaires’ disease. During human infection *Legionella* invades and grows within alveolar macrophages and epithelial cells (Steinert et al., 2002a; Köhler et al., 2003). Prevention of phagocytic killing is mediated by the type IV Dot/Icm secretion system, which delivers bacterial effector molecules that modulate the endocytic maturation of the host cell. By using *Legionella* mutants, Dictyostelium mutants, GFP-tagged bacteria and host proteins, the Dictyostelium system contributed to a roadmap of host cell factors involved in uptake and growth of *Legionella* (Fajardo et al., 2004). The details of this roadmap are discussed in the following sections.

**Establishment of the Dictyostelium–Legionella infection model**

The Dictyostelium model of Legionella infection was originally established and evaluated by analysing the intracellular growth, subcellular localization and the intracellular activities of different *Legionella* strains and *Legionella* mutants (Hägele et al., 2000; Solomon et al., 2000). This analysis revealed that virulent wild-type *L. pneumophila* grow intracellularly within membrane-bounded vacuoles of single-cell stages of *D. discoideum*. The growth rates of various well defined *Legionella* mutants like the FlaA-, Mip-, ligA-, FliA-negative mutants as well as mutants of the dot/icm genes revealed comparable results in *D. discoideum*, amoebae and macrophages (Solomon et al., 2000; Heuner et al., 2002; Steinert et al., 2002b). These findings suggest that Dictyostelium is a representative host model system which is suitable to analyse specific cellular aspects of *L. pneumophila* infection (Table 1).

**Uptake of Legionella**

Phagocytosis assays with specific cellular inhibitors and the effects of well defined host cell mutants demonstrated that *Legionella* uptake by Dictyostelium cells occurs by conventional phagocytosis which includes heterotrimeric G proteins and the phospholipase C (PLC) pathway (Fajardo et al., 2004). Additionally, these experiments revealed that cytoplasmic calcium levels, cytoskeleton-associated proteins (coronin, α-actinin/filamin, Daip1, Lim C/D, Villidin) and the calcium-binding proteins of the endoplasmic reticulum (ER), calreticulin and calnexin, specifically influence the uptake of *Legionella* (Table 1) (Müller-Taubenberger et al., 2001; Fajardo et al., 2004). Confocal microscopic time series with GFP-tagged calnexin and calreticulin demonstrated the accumulation of both pro-
teins in the phagocytic cup of *L. pneumophila*-infected host cells (Fig. 1A). These calcium-binding proteins also decorated the replicative vacuole of *L. pneumophila* at later stages of infection, while the association of these proteins with the phagocytic cup of *Escherichia coli* was transient. The exact role of calnexin and calreticulin remains to be elucidated; however, it is speculated that both proteins may have an influence on spatial calcium concentrations or the proper folding of proteins involved in infection.

**Establishment of the Legionella replicative vacuole**

The comparison of infected macrophages and *Dictyostelium* cells by transmission electron microscopy demonstrated that both cell types harbour engulfed legionellae within organelle-studded vacuoles that are associated with rough ER (Fig. 1B). As *L. pneumophila* replicates normally in certain *D. discoideum* macroautophagy mutants, the biogenesis of replicative vacuole is rather governed by other yet unidentified means (Otto et al., 2004). Colocalization studies with GFP-tagged bacteria and antibodies directed against specific lysosomal markers (DdLIMP) of *Dictyostelium* corroborated that *Legionella* inhibits the endosomal maturation pathway. Thirty minutes after infection, internalized *L. pneumophila* did not colocalize with a monoclonal antibody staining the V-ATPase of the host cell. The alternative sigma factor FliA and bacterial factors which are secreted by the Icm/Dot

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**Fig. 1.** *L. pneumophila* infection of *D. discoideum.*
A. Confocal in vivo monitoring of phagocytic cup formation (arrows) during *L. pneumophila* uptake by the host cell. The green fluorescence indicates the GFP-tagged host protein calreticulin.
B. Transmission electron micrograph of *L. pneumophila* within an early phagosome. Multiple layers of rough endoplasmatic reticulum (arrow) are associated with the phagosome.
C. Confocal in vivo monitoring of GFP-tagged host protein calreticulin (arrow) and rhodamine-labelled *L. pneumophila*. The bacteria are surrounded by calreticulin.
D. Transmission electron micrograph of a host cell nearly taken over by a bacterium-filled phagosome. Size bars as indicated.
type IV secretion system are obviously necessary to initiate this hallmark of Legionella virulence (Hilbi et al., 2001; Heuner and Steinert, 2003; Molofsky and Swanson, 2004). Multiple dot mutants of L. pneumophila (dotH, dotI, dotO) were unable to grow intracellularly in Dictyostelium or on the other hand permitted Dictyostelium growth on bacterial lawns (dotA, dotB, icmVWX, dotE, dotG, dotH, dotO) (Solomon et al., 2000). Current issues such as the contribution of the ER to bacterial survival (Roy and Tilney, 2002), or the intracellular replication of L. pneumophila within lysosomes of macrophages may be solved by applying the Dictyostelium infection model.

Intracellular growth and release of Legionella

Within the reprogrammed, maturation-blocked vacuole, transmissive L. pneumophila differentiate into a highly replicative form. This tightly regulated process is characterized by the reciprocal expression of multiple traits (Molofsky and Swanson, 2004). The responsible host signals for the differentiation are not known. However, certain host cell factors that positively influence growth rates of L. pneumophila in D. discoideum have been identified (Fig. 1C). These are the calcium-binding proteins calreticulin and calnexin, the cytoskeleton-associated proteins α-actinin/ABP120, LimC/LimD (cytoskeleton–membrane interface proteins), villidin (F-actin associated protein that associates with the Golgi apparatus and the ER), myoAl B myosin I, profilin (G actin sequestering protein), and comitin (located on vesicle and Golgi membranes) (Hägele et al., 2000; Solomon et al., 2000; Schreiner et al., 2002; Fajardo et al., 2004).

Especially interesting is the fact that Nramp1 negative Dictyostelium cells displayed a reduced phagocytosis and a better intracellular growth of L. pneumophila (B. Perachino et al. unpublished). Certain variants of this cation transporter protein influence the susceptibility of mice to parasitic infections and predispose humans for tuberculosis and leprosy (Bellamy, 1999). Whether this effect is due to changes of intraphagosomal iron concentrations is currently under investigation.

Several studies with human macrophages and various Acanthamoeba species suggest that L. pneumophila uses different mechanisms for killing and exiting mammalian and protozoan host cells (Swanson and Hammer, 2000). At 12 h post infection 71% and 74% of the L. pneumophila containing phagosomes are disrupted within macrophages and Acanthamoeba polyphaga respectively, while the plasma membrane remains intact (Molmeret et al., 2004). It is likely that the secreted phospholipases PlcA and PlaA or the cell-associated phospholipase PlaB contribute to the disruption of the phagosome (Fieger et al., 2004). In macrophages, L. pneumophila induces apoptosis and necrosis of which the latter involves pore-forming cytotoxicity (Zink et al., 2002). In protozoan cells, Legionella is either released by a pore-forming activity which kills the host or by exocytotic vesicles. Recently, it has been described that the Legionella effectors LepA and LepB, which are delivered to the host cell by the icm/Dot system, may commandeer the non-lytic protozoan exocytotic pathway (Chen et al., 2004). The analysis of the final stage of the infectious cycle in D. discoideum is still in its infancy (Fig. 1D). Microscopically it was demonstrated that one exit of L. pneumophila is the lysis of the host cell (Hägele et al., 2000). Although D. discoideum is able to undergo some kind of apoptosis, this mechanism appears not to be involved. The availability of D. discoideum exocytosis mutants, however, may help to elucidate the protozoan exocytotic pathway for dissemination of the pathogen.

Conclusion and future perspectives

This review describes different applications of Dictyostelium as host model for pathogenesis. For the further development of the Dictyostelium model in pathogenesis research, it will be important to consider that Dictyostelium does not survive temperatures above 27°C. For some pathogens which express their virulence traits at higher temperatures this limitation may be critical. Similar to mammalian cells, we also have to be aware of the substantial redundancy of cellular traits in Dictyostelium (e.g. actin-binding proteins) with multiple proteins sharing overlapping functions. As long as future approaches take these inherent limitations into account the Dictyostelium host model will undoubtedly provide an increased flexibility for novel experimental designs. Moreover, functional genomic and proteomic analysis will contribute to a very comprehensive view on both partners of a pathogenic interaction (Farbrother et al., 2002; Eichinger and Noegel, 2003). The availability of high-throughput techniques already paved the way for transcriptional studies and the elaboration of protein linkage maps. Another new approach may be the cryoelectron tomography of infected Dictyostelium cells (Medalia et al., 2002). This noninvasive three-dimensional imaging technique bridges the gap between cellular and molecular structural studies and may help to further refine our knowledge of cellular microbiology.

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