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War of the microbial worlds: Who is the beneficiary in Acanthamoeba-bacterial interactions?

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Minireview

War of the microbial worlds: Who is the beneficiary in *Acanthamoeba*–bacterial interactions?

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ABSTRACT

Acanthamoeba hosts diverse microbial organisms including viruses, bacteria, yeast and protists, some of which are potential human pathogens. The precise nature of this symbiosis is not clear, but it is suggested that such interactions enable pathogenic microbes to survive hostile conditions and lead to their transmission to susceptible hosts to establish infection. In particular, *Acanthamoeba*–bacteria interactions have gained significant attention by the scientific and the medical community and have led to speculations of employing anti-amoebic approaches in eradicating 'superbugs' from clinical settings. Here, we discuss the nature of these convoluted interactions and the benefit they represent for the symbionts.

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1. Introduction

During July 21–24, 1976, the Bellevue-Stratford Hotel Philadelphia, USA hosted the 58th State convention of the American Legion Department of Pennsylvania. When the participants and their families returned home, many of them were sick. Within days, a mystery disease had killed 29 and sickened 182 people, some of them never entered the hotel but watched a parade outside the hotel. People generally developed pneumonia-like symptoms with a cough, headache, and a high temperature. However, later stages were much more aggressive and the patients were hospitalised for weeks to months. At the time, the disease responsible for so much suffering was a mystery killer to the medical community and it was given an identity linked to those it had affected the most, hence it was called Legionnaires' disease.

At the time, no one knew of how and why so many people were getting sick or dying. All sorts of questions were raised with the quality of air within the Bellevue-Stratford hotel, the abundance of pigeon droppings outside the hotel, and the water supplies that were held in canisters throughout the convention. In December 1976, McDade determined that the aetiology of the outbreak was *Legionella pneumophila* (McDade et al., 1977; Fraser et al., 1977). In April 1977, the Centers for Disease Control, USA (http://www.cdc.gov/) officially named the disease as "Legionnaires' disease". The ubiquitous presence of *Legionella* spp. bacteria in the environment was known by 1980, and by 1981 it was known that the bacteria were found in man-made aqueous environments (Tobin et al., 1981a,b). However, how the *Legionella* spp. bacterium survived and grew in low-nutrient waters was unclear, in view of the fastidious nutritional requirements of the bacterium when cultivated on artificial media. Tim Rowbotham, a public health microbiologist in Leeds, made pivotal observations on the growth of *L. pneumophila* in free-living amoebae based on the microscopic observations of the bacteria interacting with *Acanthamoeba* (Rowbotham, 1980). Rowbotham

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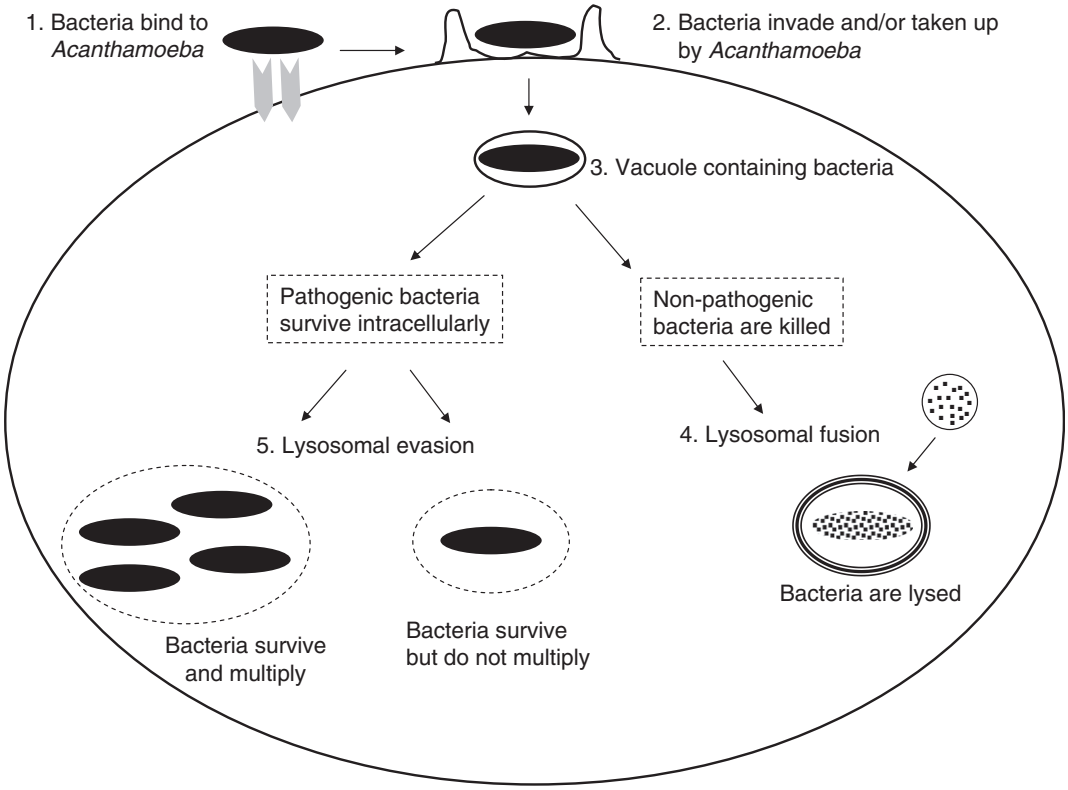

Fig. 1. Bacteria invade into and/or taken up by *Acanthamoeba*. Once intracellular, non-pathogenic bacteria are killed and used as food source, whilst the pathogenic bacteria evade intracellular killing mechanisms, and either survive or multiply within *Acanthamoeba*.

(1980) hypothesised that inhalation of *Legionella*-infected amoebal cysts is the means of transmission of Legionnaires' disease. Later, several lines of evidence explained that environmental *Legionella* spp. is dependent on the presence of *Acanthamoeba* for their growth and persistence. This served as an explanation for the growth of the bacteria in otherwise nutrient-poor water (Fields et al., 1984; Wadowsky et al., 1988) and led to the concept of "parasite-parasite interactions" ultimately leading to Legionnaires' disease.

Previous studies (1954) had shown that *Acanthamoeba* can be infected and lysed by bacteria (Drozanski, 1956), and that they harbour bacteria as endosymbionts in 1975 (Proca-Ciobanu et al., 1975) as well as act as a reservoir for pathogenic facultative mycobacteria in 1978 (Krishna-Prasad and Gupta, 1978). However, it is puzzling that *Acanthamoeba* can host bacteria and at the same time it feeds on bacteria in the environment for its nutritional requirements. Such parasite–parasite interactions are highly complex and dependent on the virulence of *Acanthamoeba*, the virulence of bacteria, and the environmental conditions. The outcome of these convoluted interactions may be beneficial to *Acanthamoeba* or to bacteria or may result in the development of a symbiotic relationship between bacteria and its *Acanthamoeba* host (Fig. 1).

2. *Acanthamoeba* as a bacterial predator

Bacteria that are non-pathogens comprise this group. In nutrient-poor water, these bacteria are generally used as a food source by *Acanthamoeba*. In such settings, bacteria are taken up by *Acanthamoeba* via phagocytosis, followed by their lysis in the phagolysosomes and benefiting the host amoeba for its nutritional needs.

3. *Acanthamoeba* as a bacterial Trojan horse

Pathogenic bacteria comprise this group. The bacteria invade *Acanthamoeba* (as opposed to taken up by amoeba) and modulate intracellular trafficking pathways to evade lysosomal killing (reviewed in Greub and Raoult, 2004). Bacteria remain viable intracellularly in *Acanthamoeba* and are transported to susceptible hosts. Upon favourable conditions (i.e., nutrient-rich), the bacteria emerge and reproduce (Alsam et al., 2006). The increasing bacterial densities lyse their host amoeba and infect new amoebae or produce disease. In the short-term, these symbiotic *Acanthamoeba*–bacteria interactions can be considered as "commensalism" where the host amoeba neither benefits nor is harmed. However, in the long-term, these interactions are "parasitic" as the result generally is the amoeba death. Given the complexities of such interactions and variable outcomes, the underlying molecular mechanisms are diverse that are regulated by parasitic bacteria (Alsam et al., 2006). The bacterial virulence determinants responsible for (i) bacterial invasion of *Acanthamoeba*, (ii) their intracellular survival, i.e., inhibition of phagolysosomes formation or growth at acidic pH, and (iii) escape from *Acanthamoeba* vary between different bacteria. The term "Trojan horse" is used to describe bacterial presence inside *Acanthamoeba* as opposed to "carrier" which may be mere attachment/adsorption on the surface. Overall, this suggests that *Acanthamoeba* facilitate bacterial survival in harsh environments and/or transmission to the susceptible hosts.

4. *Acanthamoeba* as a bacterial reservoir

Pathogenic bacteria comprise this group. Post-invasion, bacteria modulate intracellular trafficking pathways to evade the amoeba's lysosomal killing. The use of *Acanthamoeba* as a bacterial reservoir is an important property as bacterial pathogens not only survive intracellularly but also multiply within *Acanthamoeba*. This allows bacteria to survive harsh environments during transmissions from one host to another. The ability of *Acanthamoeba* to act as bacterial reservoir has gained the most attention by the scientific and medical community (reviewed in Greub and Raoult, 2004). This is due

to the fact that the majority of these bacteria are human pathogens. These include *L. pneumophila* (causative agent of Legionnaire's); *Escherichia coli* O157 (causing diarrhoea); *Coxiella burnetii* (causing Q fever); *Pseudomonas aeruginosa* (keratitis); *Vibrio cholerae* (causing cholera); *Helicobacter pylori* (gastric ulcers); *Listeria monocytogenes* (Listeriosis); and *Mycobacterium avium* (respiratory infections).

5. *Acanthamoeba* as a training ground to develop bacterial immune evasion strategies

The ability of pathogenic bacteria to survive and multiply inside *Acanthamoeba* has been hallmarked as a learning tool by bacteria to evade the onslaught of macrophage-mediated killing. This is due to the remarkable resemblance in the way numerous bacteria infect and multiply inside human macrophages and *Acanthamoeba*; not least, by using the same mechanisms at the transcriptional-, post-transcriptional- and cellular-levels indicating that amoebae and human macrophages have comparable properties that allow the bacteria to carry out their infection in both hosts. For example, the viability and intracellular growth of *L. pneumophila* within *Acanthamoeba* and human macrophages is shown to be dependent on corresponding genes including *icmT*, *icm*R, *icm*Q, *icm*P, *icm*O, *icm*M, *icm*L, *icm*K, *icm*E, *icm*C, *icm*D, *icm*J and *icm*B (Segal and Shuman, 1999). The Dot/Icm type IV secretion system is required for intracellular proliferation within human macrophages and *Acanthamoeba* (Al-Khodor et al., 2008) by evading the default endocytic pathway in both hosts (Segal and Shuman, 1999). At the same time, absence of the heavy metal efflux gene island in *L. pneumophila* is required neither for survival nor replication inside *A. castellanii* or human macrophages (Kim et al., 2009).

The ability of *Acanthamoeba* to harbour a variety of microbes including viruses (Mimivirus, coxsackieviruses, adenoviruses, poliovirus, echovirus, enterovirus, vesicular stomatitis virus, etc.), bacteria (*Aeromonas*, *Bacillus*, *Bartonella*, *Burkholderia*, *Campylobacter*, *Chlamydophila*, *Coxiella*, *E. coli*, *Flavobacterium*, *Helicobacter*, *Legionella*, *Listeria*, *Staphylococcus*, *Mycobacteria*, *Pasteurella*, *Prevotella*, *Porphyromonas*, *Pseudomonas*, *Rickettsia*, *Salmonella*, *Shigella*, *Vibrio*, etc.), protists (*Cryptosporidium*, *Toxoplasma gondii*) and yeast (*Cryptococcus*, *Blastomyces*, *Sporothrix*, *Histoplasma*, *Streptomyces*, *Exophiala*, etc.) (reviewed in Khan, 2009) suggests that we can no longer focus on a single aetiological agent, where the link between exposure and infection is clearly defined. For the development of preventative and possibly therapeutic measures in such cases, the disease should be considered as having multiple and confounding factors, with different aetiologies specific to the different affected subpopulations, in order to understand fully the dynamics of disease outbreaks. With the remarkable implications of parasite-parasite interactions, which may contribute to the evolution of one or both parasites to become successful human and animal pathogens, this area of research, i.e., "war of the microbial worlds" will continue to generate increasing attention from the scientific and the medical community in the future.

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