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Review

The *in vivo* extracellular life of facultative intracellular bacterial parasites: Role in pathogenesis

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In memory of Manuel Teixeira da Silva (April 10th 2012). *Scientist extraordinaire, savvy, kind-hearted and gentle scholar, who didn't like tributes but left such a strong mark in our hearts and inquisitive minds. Great devotee of E. Metchnikoff, in whose work, meticulously studied, he found great inspiration and knowledge.*

"Any path to knowledge is a path to God – or Reality, whichever word one prefers to use." Arthur C. Clarke.

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ABSTRACT

Classically labeled facultative intracellular pathogens are characterized by the ability to have an intracellular phase in the host, which is required for pathogenicity, while capable of extracellular growth *in vitro*. The ability of these bacteria to replicate in cell-free conditions is usually assessed by culture in artificial bacteriological media. However, the extracellular growth ability of these pathogens may also be expressed by a phase of extracellular infection in the natural setting of the host with pathologic consequences, an ability that adds to the pathogenic potential of the infectious agent. This infective capability to grow in the extracellular sites of the host represents an additional virulence attribute of those pathogens which may lead to severe outcomes. Here we discuss examples of infectious diseases where the *in vivo* infective extracellular life is well documented, including infections by *Francisella tularensis*, *Yersinia pestis*, *Burkholderia pseudomallei*, *Burkholderia cenocepacia*, *Salmonella enterica* serovar Typhimurium and *Edwardsiella tarda*. The occurrence of a phase of systemic dissemination with extracellular multiplication during progressive infections by facultative intracellular bacterial pathogens has been underappreciated, with most studies exclusively centered on the intracellular phase of the infections. The investigation of the occurrence of a dual lifestyle in the host among bacterial pathogens in general should be extended and likely will reveal more cases of infectious diseases with a dual infective intracellular/extracellular pattern.

Contents

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Introduction

Symbiotic associations include mutualism, commensalism and parasitism. In microbiology, parasitism implies that the microbe produces disease in its host, although it may have separate ways of life like survival or replication in the environment. Thus, pathogens are infective when they are associated with their hosts and live producing host damage. Important microbial attributes that confer the potential for virulence include the ability to enter a host, to evade host defenses, to adapt to and grow in a host environment and to counteract host immune responses leading to a successful infection, a pathological inflammatory response to the disease-causing presence of microorganisms in normally sterile host tissue (Bone et al. 1992; Casadevall and Pirofski 2009). Thus, the damaging result of the presence of a microbe within a host is dependent on the characteristics of the pathogen and the host (Finlay and Falkow 1989; Casadevall and Pirofski 2001), and the full pathogenetic potential of a microbe is achieved when it expresses the relevant virulence factors and infects a highly susceptible, untreated host. The outcome of the infectious process will vary according to the balance between microbial virulence and host resistance and may reach serious consequences including the death of the host. These concepts should be taken into account when analyzing progressive, successful infections. The present text discusses relevant pathogenic aspects of the extracellular life stage of some facultative intracellular bacterial pathogens when advantage of the pathogen's virulence attributes over the host immunocompetence is present. This ability to develop an extracellular phase of infection gives additional infectious advantages to the pathogen. In some cases, this extracellular stage involves systemic dissemination and multiplication and may be responsible for the progression and increased severity of the disease, as will be here discussed.

Extracellular and intracellular parasitism as distinct lifestyles of bacterial pathogens

Classically, infectious agents are classified as extracellular or intracellular pathogens, and the latter have been subdivided in facultative and obligate.

When producing disease, extracellular pathogens typically multiply in the host extracellular territories like mucosal surfaces, vascular and lymphatic systems and other body cavity fluids and interstitial spaces (Stuart and Ezekowitz 2005). They use a plethora of virulence mechanisms including to evade the antimicrobial capabilities of phagocytosis thus promoting extracellular multiplication (Weiser and Nahm 2008). *Staphylococcus aureus*, *Streptococcus pyogenes*, *Pseudomonas aeruginosa*, and *Escherichia coli* are typical examples of bacteria which have been labeled extracellular pathogens and wound infections, osteomyelytis, scarlet fever, certain forms of pneumonia, urinary tract infections are examples of infections caused by these pathogens.

Classical examples of intracellular pathogens are *Brucella abortus*, *Listeria monocytogenes*, *Chlamydia trachomatis*, *Coxiella burnetii*, *Mycobacterium tuberculosis*, *Salmonella enterica* and typical infectious diseases caused by them include brucellosis, listeriosis, tuberculosis and salmonellosis. These pathogens have the ability to establish a relationship in the susceptible host which includes a stage of intracellular multiplication (Suter 1956; Moulder 1985), frequently within macrophages (Pamer 2008). To become intracellular and maintain an intercellular phase, these pathogens promote the entry into host cells (Sansonetti 2001; Cossart and Sansonetti 2004), escape or resist cellular antimicrobial mechanisms, adapt to the new environment and then modulate host cell biology to create a novel intracellular replication niche (Brubaker 1985). As will be discussed in detail later, such intracellular stage is crucial for the pathogenicity of these bacteria (Kaufmann 1999; Bogdan 2008), a central issue that has frequently been neglected.

The classical division of intracellular pathogens in facultative and obligate has been based on the presence (in facultative intracellular bacteria) or absence (in obligate intracellular bacteria) of the capacity to multiply in a cell-free environment (Suter 1956; Moulder 1985). This feature usually is evaluated by the ability to multiply in artificial bacteriological media (Moulder 1985; Renesto et al. 2003; Toft and Andersson 2010). However, the use of artificial cell-free culture media which has been invaluable for the isolation of causative infectious agents and the realization of essential experimental work does not reproduce the *in vivo* conditions prevailing during a real infectious disease.

We will discuss several examples of bacterial infections where an extracellular multiplication in host territories of intracellular pathogens represents a crucial phase in the microbe's infectious life cycle. Diverse facultative intracellular bacterial pathogens have briefly been reported to exhibit the dual style of infectivity showing in the host an intracellular/extracellular microbiological behavior. These include, among others, *L. monocytogenes* (Hof et al. 1997), *Brucella* sp. (Anderson et al. 1986), *Erysipelothrix rhusiopathiae* (Bender et al. 2009), *Tropheryma whipplei* (Renesto et al. 2003), *Bartonella quintana* (Benslimani et al. 2005) and *Shigella* (or *E. coli* pathovar EIEC (Lan and Reeves 2002; Croxen and Finlay 2010)). However, the extracellular behavior of most of these pathogens has not been fully analyzed and the relevance for pathogenicity of their extracellular phases in the host is not yet acceptably clarified. These uncertainties prompt for further research.

M. tuberculosis is a paradigmatic example of facultative intracellular pathogens and the intracellular phase of its growth in the host has been extensively studied (Bru and Cardona 2010). In the life cycle of *M. tuberculosis* phases of extracellular residence and replication occur and have a role in the pathogenesis and transmission of tuberculosis. Seminal studies by Dannenberg have highlighted the importance of the extensive extracellular multiplication of *M. tuberculosis* in advanced progressive pulmonary tuberculosis (Dannenberg 1994a,b). These studies have shown that the caseous centers formed in advanced lesions may liquefy providing an environment favorable to the extensive extracellular replication of the pathogen which may reach huge numbers. From these foci, the bacilli spread to other parts of the lung and to the environment promoting the disease transmission. As in other situations here discussed, the extension of the extracellular multiplication of *M. tuberculosis* is greater in immunocompromised mice and may be associated with terminal disease (Kramnik et al. 2000).

Among the examples of facultative intracellular pathogens where an extracellular phase of infection has been well documented, we selected a group of proteobacteria that includes *Francisella tularensis*, *Yersinia pestis*, *Burkholderia pseudomallei*,