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Review

Metabolic adaptation of intracellular bacteria and fungi to macrophages

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ABSTRACT

The mature phagosome of macrophages is a hostile environment for the vast majority of phagocytosed microbes. In addition to active destruction of the engulfed microbes by antimicrobial compounds, restriction of essential nutrients in the phagosomal compartment contributes to microbial growth inhibition and killing. However, some pathogenic microorganisms have not only developed various strategies to efficiently withstand or counteract antimicrobial activities, but also to acquire nutrients within macrophages for intracellular replication. Successful intracellular pathogens are able to utilize host-derived amino acids, carbohydrates and lipids as well as trace metals and vitamins during intracellular growth. This requires sophisticated strategies such as phagosome modification or escape, efficient nutrient transporters and metabolic adaptation. In this review, we discuss the metabolic adaptation of facultative intracellular bacteria and fungi to the intracellular lifestyle inside macrophages.

1. Antimicrobial mechanisms of macrophages

Macrophages are phagocytic cells of the innate immune system patrolling in nearly all human tissues and on mucosal surfaces and thus contributing to the first line of defence against invading microbes (Pollard, 2009). Defined microbe-associated molecular patterns (MAMPs) of invading microorganisms are recognized by vesicular or cytosolic pattern recognition receptors (PRRs) of macrophages (reviewed by (Erwig and Gow, 2016; Ren et al., 2017; Weiss and Schaible, 2015)) leading to host signalling cascades, which regulate downstream processes such as immune cell activation, changes in host cell metabolism, inflammation and phagocytosis (Ren et al., 2017; Weiss and Schaible, 2015). Phagocytosis represents a cellular uptake machinery required for efficient destruction of invading microbes (reviewed in (Mao and Finnemann, 2015)). Following phagocytosis, ingested microbes are localized within defined membrane-enclosed phagosomal compartments, which mature to phagolysosomes *via* a tightly regulated series of fusion events with the endocytic machinery (Jeschke and Haas, 2016; Mao and Finnemann, 2015; Pauwels et al., 2017; Stein et al., 2012). In the maturated phago(lyso)some, microbes are exposed to an acidic hostile environment with antimicrobial activities due to lysosomal proteases, reactive oxygen species and antimicrobial peptides (Flannagan et al., 2015). Nevertheless, an astonishing number of microbes have developed strategies to survive within this intracellular compartment.

Intracellular survival strategies are manifold and extensively reviewed elsewhere (Awuh and Flo, 2017; Gilbert et al., 2014; Ramond et al., 2012; Sarantis and Grinstein, 2012). Some microbes adapt to antimicrobial activities within the phagosome, while others either actively modify the phagosome, inhibit phagosome maturation (thereby generating a less hostile replicative environment), or escape from the phagosomal compartment into the cytoplasm.

In addition to the above-mentioned direct antimicrobial mechanisms, phagocytosed pathogens also face an environment with a reduced supply of nutrients as compared to the extracellular milieu. For example, essential trace metals like iron or manganese and other nutrients are scarce due to host induced sequestration activities (Appelberg, 2006; Flannagan et al., 2015). Thus, microbial growth inhibition or killing within phagocytes may not only be based on the toxic environment in the phagosome, but may also result from the scarcity of

Abbreviations: BCAA, branched chain amino acids; CAT, cationic amino acid transporter; COX, cytochrome c oxidase; ED, Entner-Doudoroff; EMP, Embden-Meyerhof-Parnas; GlcNAc, *N*-acetyl-glucosamine; GM-CSF, granulocyte macrophage colony-stimulating factor; IL-4, interleukin 4; IFNγ, interferon-γ; LCV, *Legionella*-containing vacuole; MAMPs, microbe-associated molecular patterns; PLC, phospholipase C; PP, pentose phosphate; PRR, pattern recognition receptors; ROS, reactive oxygen species; SCV, *Salmonella*-containing vacuole; SIF, *Salmonella*-induced filaments; SPI, *Salmonella*-pathogenicity island; SOD, superoxide detoxifying enzyme; TCA, tricarboxylic acid; T3SS, type III secretion system

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nutrients. Consequently, successful facultative intracellular pathogens must not only be equipped with strategies that ensure avoidance of or resistance to toxic mechanisms of macrophages, but also be able to adapt to the special nutritional conditions inside macrophages.

In this review, we focus on the impact of nutrients within macrophages for intracellular pathogens, as well as on the adaptation of pathogens to these nutritional conditions. We discuss strategies of selected examples of bacterial and fungal pathogens with diverse infection cycles including stages of intracellular proliferation within macrophages. In addition to macrophages, we discuss intracellular survival within amoebae (e.g. *Acanthamoeba castellanii*). Amoebae show macrophage-like behaviour and microbicidal activity (Guimaraes et al., 2016), and successful environmental mammalian pathogens, such as *Legionella pneumophila* or *Cryptococcus neoformans,* are known to have the potential to resist antimicrobial activities and to replicate intracellularly in environmental amoebae (Guimaraes et al., 2016). Selected exemplary bacterial and fungal pathogenic species discussed in this review are briefly introduced in the following paragraphs.

Mycobacterium tuberculosis, a Gram-positive bacterium with an exceptional hydrophobic cell wall, is the causative agent of tuberculosis and has evolved strategies to use macrophages as a replicative niche, thus evading host immune responses and allowing it to persist and/or spread in the host (Awuh and Flo, 2017). *M. tuberculosis* prevents phagosome-lysosome fusions and efficiently manipulates host macrophages to obtain sufficient host-derived nutrients to ensure survival, replication and intracellular persistence (Neyrolles et al., 2015).

The Gram-negative bacterium *Legionella pneumophila*, etiological agent of severe pneumonia (Legionnaire's disease), replicates in environmental amoebae, accidentally infects humans and resists human alveolar macrophages after phagocytosis (Khodr et al., 2016). Inside macrophages, *L. pneumophila* induces the formation of a replicative non-endosomal vacuole, where the pathogen is able to acquire nutrients, replicate and further differentiate into a flagellated form, which mediates escape from the phagosome into the cytoplasm and finally from the macrophage into the extracellular space (Eisenreich and Heuner, 2016; Robertson et al., 2014).

Francisella tularensis is a Gram-negative, highly virulent, zoonotic bacterium, which can infect a broad range of mammalian species and cause tularemia (Meibom and Charbit, 2010). During infection, this facultative intracellular bacterium primarily infects macrophages and enters the phagosomal compartment, but to survive and replicate within infected macrophages, *F. tularensis* needs to escape from the phagosome into the cytosol (Ramond et al., 2012).

Listeria monocytogenes is a facultative intracellular Gram-positive bacterium that causes the fatal foodborne disease listeriosis (Vazquez-Boland et al., 2001). After phagocytosis by macrophages, *L. monocytogenes* prevents the maturation of the phagosome, escapes to and replicates within the cytosol (Ireton et al., 2014).

Salmonella enterica is a facultative intracellular Gram-negative pathogen that causes a range of diseases in humans. *S. enterica* serovars such as Typhimurium or Enteritidis are frequently transmitted from reservoirs in livestock and cause gastroenteritis, a self-limiting infection of the small intestine with local inflammation. High human-adapted serovars Typhi and Paratyphi A are transmitted from infected humans and cause a systemic, often fatal disease termed typhoid fever. The ability of certain *Salmonella* spp. to survive and replicate within phagocytic cells is generally considered as requirement to cause systemic diseases (Fields et al., 1986). However, this notion is mainly based on experimental studies in a systemic infection model with *S.* Typhimurium in susceptible mouse strains.

Candida albicans and *C. glabrata* are commensal yeasts of warm-blooded animals and humans as well as opportunistic fungal pathogens that provoke superficial mucosal infections, but also life-threatening systemic candidiasis (Odds, 1988). Both *Candida* species can resist elimination by macrophages and have evolved strategies to proliferate within phagocytes (Erwig and Gow, 2016; Gilbert et al., 2014; Kasper et al., 2015). Phagocytosis of *C. albicans* by macrophages induces intracellular filamentation, which mediates damage of the host cell and escape, whereas *C. glabrata* is not able to form hypha and grows in the phagosome as yeast cells until the host macrophages burst (Miramon et al., 2013; Seider et al., 2010).

The encapsulated and opportunistic fungus *Cryptococcus neoformans* is able to cause life-threatening respiratory and systemic infections (cryptococcosis) in immunocompromised individuals (Johnston and May, 2013). Inhaled airborne yeast cells, or sexually produced basidiospores, of environmental *Cr. neoformans* switch to capsulated yeast morphology (Kwon-Chung et al., 2014). Although capsules inhibit phagocytosis by alveolar macrophages, *Cr. neoformans* is known to persist and proliferate within a mature phagolysosome once phagocytosed (Gilbert et al., 2014; Johnston and May, 2013; Leopold Wager et al., 2016).

The environmental fungal pathogen *Histoplasma capsulatum*, causing most endemic respiratory mycoses in the USA, is able to infect macrophages, resist the antimicrobial activities and proliferates intracellularly (Garfoot and Rappleye, 2016; Horwath et al., 2015). Engulfed by macrophages, *H. capsulatum* enters the phagosomal compartment, blocks acidification and avoids killing e.g. due to detoxification of reactive oxygen radicals (Gilbert et al., 2014).

2. Nutrient limitation as an antimicrobial strategy

Phagocytosis by macrophages is thought to generate an environment with restricted nutrient availability as compared to the extracellular milieu, and there is evidence that macrophages actively deprive pathogens of accessible nutrients (Appelberg, 2006). However, intracellular pathogens have developed specific virulence mechanisms that target host biosynthetic and degradation pathways or nutrient-rich sources to enhance supplies of limiting nutrients, a paradigm termed as 'nutritional virulence' (Abu Kwaik and Bumann, 2013). However, the defined characterization of the biochemical composition of pathogen-containing compartments in macrophages has been challenging, and knowledge about nutrient composition has mostly been derived indirectly from microbial responses to conditions in their intracellular niche (Appelberg, 2006; Weiss and Schaible, 2015). For example, it has been assumed from transcriptional profiling approaches and metabolic flux analysis that intraphagosomal pathogens encounter glucose and trace metal limitation and rely on alternative C_2 and C_3 carbon sources like amino acids or lipid degradation products. Examples for such proposed nutritional conditions come from studies dealing with intracellular bacterial (*M. tuberculosis*) and fungal pathogens (*C. albicans*) (Lorenz et al., 2004; Lorenz and Fink, 2002; McKinney et al., 2000). However, it is unlikely that these observations can be generalized for an intracellular life style *per se*, as the replicative niche used inside macrophages differs among pathogens. For example, the cytosol, used by several intracellular bacteria, is thought to be less nutrient-deprived as compared to membrane-enclosed compartments (Appelberg, 2006). Further, the fact that *L. pneumophila* uses amino acids, glucose and glycerol as major nutrients during intra-vacuolar growth (in contrast to *M. tuberculosis* and *C. albicans*) (Eisenreich and Heuner, 2016), points to a defined nutrient composition depending on the pathogen-associated membrane-enclosed compartment. In line with this, the active manipulation of the host endocytic pathways by intracellular pathogens results in not only a less hostile niche, but in a distinct replication-permissive pathogen vacuole with rather favourable conditions including sufficient access to nutrients (e.g. *Salmonella*, *Legionella*) (Hilbi and Haas, 2012).

Due to incomplete amino acid biosynthesis clusters, pathogens like *F. tularensis* require host-derived amino acids within the intracellular milieu of macrophages (Meibom and Charbit, 2010). Interestingly, some of these amino acids, for example cysteine, are strongly limited within host cells (Meibom and Charbit, 2010). Therefore, intracellular growth of some intracellular pathogens maybe suppressed by limiting