Immunity
Perspective

The Macrophage Paradox

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Macrophages are a diverse population of phagocytic cells that reside in tissues throughout the body. At sites of infection, macrophages encounter and engulf invading microbes. Accordingly, macrophages possess specialized effector functions to kill or coordinate the elimination of their prey. Nevertheless, many intracellular bacterial pathogens preferentially replicate inside macrophages. Here we consider explanations for what we call "the macrophage paradox:" why do so many pathogenic bacteria replicate in the very cells equipped to destroy them? We ask whether replication in macrophages is an unavoidable fate that essentially defines a key requirement to be a pathogen. Conversely, we consider whether fundamental aspects of macrophage biology provide unique cellular or metabolic environments that pathogens can exploit. We conclude that resolution of the macrophage paradox requires acknowledgment of the richness and complexity of macrophages as a replicative niche.

Introduction: Macrophage Diversity

The term "macrophage" encompasses a large and heterogeneous group of tissue-resident phagocytes. In the brain, macrophage-like cells are called microglia; in the liver, they are called Kupffer cells; in the skin, they are called Langerhans cells; in the bone, they are called osteoclasts; and elsewhere, they are identified by the tissues they inhabit (e.g., peritoneal macrophages, alveolar macrophages). The diverse anatomical localization of macrophages is mirrored by their substantial phenotypic diversity and plasticity, leading some to despair there might indeed be "no such thing as a 'macrophage' " (Wynn et al., 2013).

Nevertheless, investigators have identified a core transcriptional signature characteristic of macrophages from diverse tissues (Gautier et al., 2012). This core signature includes transcripts encoding the high-affinity Fcγ receptor I and MerTK, a receptor involved in uptake of apoptotic cells, consistent with the notion that phagocytosis is a core function of macrophages. In addition, macrophages are enriched for the expression of sensor proteins, such as Toll-like receptors (TLRs), RIG-I-like receptors (RLRs), and the cytosolic nucleotide-binding domain leucine-rich repeat-containing proteins (NLRs) (Takeuchi and Akira, 2010). Upon engagement of these sensors, macrophages rapidly differentiate into robust producers of diverse chemokines and cytokines. Once activated, especially if by interferon-γ (IFN-γ), macrophages exhibit a potent capacity for killing and degrading engulfed material. Macrophages can also be activated through exposure to T helper 2 (Th2) cell-associated cytokines such as interleukin-4 (IL-4) and IL-13 to become cells that are instead optimized for resolution of inflammation and the coordination of tissue repair (Gordon, 2003; Martinez and Gordon, 2014).

The phenotypic diversity of macrophages might not be reflected by in vitro studies, which frequently rely on macrophages differentiated from mouse bone marrow cells. Such bone-marrow-derived macrophages are known to differ substantially from bona fide tissue-resident macrophages in mice and humans (Epelman et al., 2014; Gautier et al., 2012; Murray et al., 2014). In vivo, the population of macrophages present at a site of bacterial infection can derive from several sources, including self-perpetuating tissue-resident populations separated early in development from yolk sack progenitors and infiltrating monocytes differentiated from bone marrow hematopoietic stem cells (Epelman et al., 2014; Geissmann et al., 2010; Hashimoto et al., 2011; Yona et al., 2013).

Given that macrophages can encompass a wide variety of cellular phenotypes, perhaps the best way to generalize the function of macrophages is simply to state that they detect alterations—stress, infection, injury—in the tissues they inhabit and then initiate the cellular responses that return the tissues to homeostasis (Medzhitov, 2008). In fact, given their diverse functions as antigen presenters, cytokine producers, pathogen sensors, tissue restorers, and microbe killers, perhaps the most salient feature of macrophages is the extent to which they must satisfy many competing demands. Macrophages are cells that initiate inflammation and tissue destruction, but they must also initiate tissue repair. Macrophages are typically long-lived in tissues, yet as described below, infected macrophages are also able to undergo an extremely rapid form of cell death called pyroptosis. To foreshadow our conclusion, the competing demands faced by macrophages imply the existence of evolutionary trade-offs among these demands—trade-offs that we suggest microbes can readily evolve to exploit.

Antimicrobial Effector Functions of Macrophages

Killing intracellular microbes is a key function of macrophages (Flannagan et al., 2009). The antimicrobial effector functions of macrophages can be divided generally into cell-autonomous and non-cell-autonomous mechanisms, which cooperate in the goal of tissue sterilization. Cell-autonomous defenses include degradative enzymes such as proteases, nucleases, and lysozyme, which digest microbes in mature acidified phagosomes. In addition, the production of antimicrobial peptides (Nizet, 2006) and reactive oxygen or nitrogen species (Nathan and Cunningham-Bussel, 2013) can kill or damage ingested microbes.

Phagosomal bacteria, as well as bacteria that escape the phagosome, can also be targeted for elimination by selective autophagy (Randow and Youle, 2014). It is important to emphasize that although these cell-autonomous antimicrobial strategies are readily employed by macrophages, they are certainly not unique to macrophages. Thus, the inhospitality of macrophages compared with other cell types is not absolute and is instead only a matter of degree. Chemokines and cytokines produced by infected macrophages can have non-cell-autonomous effects that limit pathogen replication, via recruitment and activation of other cells in the vicinity of an infected macrophage. Nitric oxide (NO) can also act non-cell-autonomously (Olekhnovitch et al., 2014).

Another macrophage defense against intracellular pathogens is a rapid form of cell death called pyroptosis (Bergsbaken et al., 2009; Miao et al., 2010). Pyroptosis results from inflammasome-dependent activation of proinflammatory caspases such as Caspase-1 and Caspase-11. Pyroptosis is partly a cell-autonomous and partly a non-cell-autonomous form of defense. Pyroptosis is cell autonomous in the sense that it eliminates an otherwise hospitable intracellular niche for pathogen replication. However, the effectiveness of pyroptosis relies in part on a network of extracellular defenses to ultimately eliminate pathogens. Indeed, inflammasome activation leads not only to pyroptosis, but also to release of IL-1, an effective inducer of neutrophil recruitment. Neutrophils are exceptionally microbicidal cells containing high concentrations of degradative enzymes and antimicrobial peptides. In this sense, pyroptosis and neutrophils are collaborative, with the former ejecting pathogens from their protected intracellular niche, enabling the latter to close in for the kill.

Several macrophage antimicrobial defenses, particularly autophagy and reactive nitrogen intermediates, are most strongly induced in macrophages in the presence of IFN-γ. IFN-γ, and to a lesser degree type I IFNs, are able to induce antimicrobial GTPases such as p47 and GPB family members (Kim et al., 2012). Robust stimulation by IFN-γ almost invariably renders a macrophage completely inhospitable to invading microbes due to the combination of antimicrobial responses induced by this cytokine (Schroder et al., 2004). However, given the potential for collateral tissue damage, IFN-γ must be tightly controlled to maintain homeostasis and avoid autoimmunity (Pollard et al., 2013). The need to regulate IFN-γ probably limits the ability of this pathway to fully control intracellular parasitism.

The Macrophage Paradox

Although we have emphasized the diversity of macrophages, it is nevertheless clear that one of the specialized functions of macrophages is to orchestrate the elimination of microbes. Indeed, macrophages are outranked as microbial assassins only perhaps by neutrophils. Given this, we find it striking that so many intracellular bacterial pathogens replicate in macrophages. Table 1 lists most of the commonly studied bacterial pathogens that are traditionally classified as intracellular. We acknowledge that classification of a given pathogen as "intracellular" versus "extracellular" is often controversial. Many bacterial pathogens—e.g., *Pseudomonas aeruginosa*, *Yersinia spp*., *Bacillus anthracis*, etc.—spend a portion of their lives intracellularly, where they can survive and in some cases even replicate. In Table 1 we focus primarily on 17 well-studied bacterial species for which intracellular replication (and not simply intracellular survival) is a predominant or critical component of the species' pathogenic lifestyle. Of these 17 species, at least 12 have been reported to have the capacity to replicate in macrophages. In most of these cases, macrophages are a preferential host cell in vivo. These observations lead to an apparent conundrum we refer to as "the macrophage paradox" (Figure 1): why do so many bacterial pathogens replicate in macrophages, given that macrophages are a cell type that appears adapted to kill and eliminate bacteria?

Paradoxes generally arise from a seeming—but not a real or actual—contradiction. Accordingly, we propose that there are several non-mutually-exclusive explanations for why intracellular bacteria frequently occupy macrophages as an intracellular niche. In fact, we find the macrophage paradox intriguing in part because of the number of distinct explanations that can be proposed to resolve it (Figure 2). Our discussion focuses on bacterial pathogens, because a similar propensity to replicate in macrophages does not appear to exist among other classes of pathogenic microbes. This bacteria-specific nature of the macrophage paradox is discussed in more detail below.

Pathogen Adaptation to the Macrophage Niche

Before addressing various resolutions of the macrophage paradox, it is worth noting the remarkable number of different strategies pathogens have evolved in order to replicate in macrophages (Ray et al., 2009; Thi et al., 2012). Several pathogens replicate within a variety of membrane-bound compartments, typically derived from a phagosome, and frequently referred to as pathogen-containing "vacuoles." For example, *Legionella pneumophila* resides in a phagosome that at least initially resists acidification, whereas the closely related pathogen *Coxiella burnetti* appears to embrace an acidified phagosomal environment. The intracellular replicative compartments of *Salmonella enterica*, *Mycobacterium tuberculosis*, *Chlamydia pneumoniae*, and *Brucella abortus* can all be molecularly distinguished (Table 1). The virulence factors pathogens utilize to create these intracellular compartments are also varied. Pathogens alternately employ type III, type IV, type VI, or type VII secretion systems to deliver diverse, evolutionarily unrelated effectors that manipulate distinct aspects of host cell biology (Table 1). Another major class of intracellular pathogens elects to escape the membrane-bound phagosome and instead replicate within the host cell cytosol. Examples of such pathogens include *Listeria monocytogenes*, *Francisella tularensis*, and *Burkholderia pseudomallei*. Escape to the cytosol can be mediated by type III or type VI secretion systems or a variety of pore-forming toxins. Recent evidence suggests that even pathogens considered vacuolar nevertheless experience a degree of cytosolic exposure; conversely, "cytosolic" pathogens can also engage membranous compartments such as autophagosomes (Deretic, 2012; Lam et al., 2012; Watson et al., 2012). Thus, the intracellular habitat is complex and challenging for pathogens to navigate. Yet the diversity of mechanisms bacteria use to replicate in macrophages suggests both that there are many ways to penetrate the defenses of macrophages and that many pathogens have found replication in macrophages to be an evolutionary path of least (or at least low) resistance. After considering the apparent ease with which pathogens can evolve to replicate in macrophages, one might be tempted to conclude that macrophages are not only a poor defense system, but are even a particularly weak point of vulnerability.

Table 1. Replicative Niches of Intracellular Bacterial Pathogens

Name of Bacteria	Human Disease	Replicates in Macrophages?	Replicates in Other Cell Type(s)?	Intracellular Niche	Virulence Factors[a]
Anaplasma phagocytophilum	granulocytic anaplasmosis; tick-borne fever	mainly granulocytes	granulocytes and endothelial cells	membrane-bound "inclusion"	T4SS
Bartonella henselae	cat-scratch disease	no?	endothelial cells; erythrocytes in cats	membrane-bound vacuole	T4SS
Brucella abortus, melitensis	brucellosis	yes	mainly in macrophages; also placental trophoblasts	ER-like vacuole	T4SS
Burkholderia pseudomallei	melioidosis	yes	yes, including neutrophils	cytosol	T3SS; T6SS
Chlamydia pneumoniae	pneumonia	yes	yes, but mainly macrophages	membrane-bound "inclusion"	T3SS
Chlamydia trachomatis	trachoma, pelvic inflammatory disease, etc.	poorly if at all	epithelial cells	membrane-bound "inclusion"	T3SS
Coxiella burnetii	Q fever	yes	yes, but mainly professional phagocytes	phagolysosome-like compartment	T4SS
Edwardsiella tarda	rare; typically gastroenteritis	yes	yes, e.g., epithelial cells	phagosome-derived compartment	T3SS; T6SS
Ehrlichia chaffeensis	monocytic ehrlichiosis	yes	mainly monocytes and macrophages	early endosome-like "inclusion"	T4SS
Francisella tularensis	tularemia	yes	mainly macrophages? Also epithelial and other cells	cytosol	T6-like SS (FPI)
Legionella pneumophila	Legionnaires' disease	yes	mainly macrophages in mammals, but also protozoa	ER-like vacuole	T4SS
Listeria monocytogenes	gastroenteritis; bacteremia	yes	CD8α dendritic cells	cytosol	Listeriolysin O, ActA
Mycobacterium tuberculosis	tuberculosis	yes	mainly macrophages	Membrane bound compartment	T7SS (ESX)
Rickettsiae	Rocky Mountain spotted fever, typhus, etc.	yes, but mainly endothelial cells	primarily vascular endothelium	cytosol	various
Salmonella enterica	typhoid fever, gastroenteritis	yes	dendritic cells, gut epithelial cells	late endosomal compartment	T3SS
Shigella flexneri	diarrhea	poorly if at all	mainly intestinal epithelial cells	cytosol	T3SS

[a]Abbreviations are as follows: T3SS, type III secretion system; T4SS, type IV secretion system; T6SS, type VI secretion system; T7SS, type VII secretion system.

Is Replication in Macrophages Inevitable?

A trivial resolution of the macrophage paradox is the view that pathogens do not "elect" to replicate in macrophages, but instead have little choice in the matter. Under this view, it is considered inevitable that an invading pathogen will eventually find itself in a macrophage. Thus, success as a pathogen requires, at least in part, the ability to replicate in macrophages. An extreme version of this view holds that replication, or at least survival, in macrophages is an essential part of what it means to be a pathogen.

The idea that macrophages represent an inevitable destination for pathogens has some appeal. The localization of macrophages to virtually every tissue in the body means that there is essentially no site of infection in which an invading microbe would not encounter a macrophage. In addition, macrophages are professional phagocytes, optimized for engulfment of particles, cellular debris, apoptotic cells, and, of course, microbes. If a pathogen does not evolve a specific mechanism to enter another cell type or avoid phagocytosis, it is likely that the pathogen will soon find itself engulfed by a macrophage. Pathogens that preferentially invade nonmacrophage cells might nevertheless find themselves in a macrophage if their primary host cell undergoes apoptosis and subsequent phagocytosis by a nearby macrophage. Moreover, even the most ardent and devoted intracellular pathogens experience at least part of their life cycle in the extracellular space and are thus subject to uptake into macrophages.

Despite these considerations, we believe that the overall balance of evidence favors the view that replication in macrophages is *not* simply inevitable, but instead most frequently reflects a strategic "choice" made by pathogens that is more appealing than other options. Indeed, if uptake by a macrophage is inevitable, then replication in neutrophils might be considered even more so, especially if macrophage pyroptosis serves to transfer pathogens from macrophages to neutrophils. Neutrophils swarm to sites of infection in large numbers and are highly phagocytic. Yet few bacterial pathogens are known to replicate efficiently in neutrophils. As discussed above, this is almost certainly because neutrophils express abundant antibacterial enzymes that make the neutrophil a particularly toxic environment for replication. Indeed, neutropenic humans and mice are highly susceptible to bacterial infections, illustrating the extent to which neutrophils

Figure 1. The Macrophage Paradox
Why do so many bacterial pathogens make macrophages, a menacing cell type, their home? Illustration by Kyle Gabler.

provide a major barrier to bacterial infection. Moreover, neutrophils are notoriously short-lived cells that do not provide a stable replicative niche. *Anaplasma phagocytophilum* is a fascinating and rare example of a pathogen with the dedicated capacity to replicate in neutrophils (Rikihisa, 2010), but *A. phagocytophilum* might be the exception that proves the rule. For most pathogens, the replicative calculus favors macrophages as a kinder, gentler, and longer-lived host cell, not simply an inevitable niche.

Underlining the optionality of the macrophage niche is the existence of several bacterial pathogens that do not replicate in macrophages (Table 1). *Shigella flexneri* and *Chlamydia trachomatis* are examples of intracellular bacterial pathogens that are largely able to avoid macrophages by replicating in epithelial cells. *Rickettsia* species appear to prefer to replicate in vascular endothelial cells (Mansueto et al., 2012). Numerous human bacterial pathogens—for example, *Vibrio cholerae*, *Yersinia pestis*, *Bacillus anthracis*, and *Staphylococcus aureus*—primarily replicate extracellularly and are able to resist phagocytosis by deploying virulence factors such as capsules or toxins (Sarantis and Grinstein, 2012). The variety of nonmacrophage replicative niches and the seemingly unlimited inventiveness with which bacterial pathogens are able to exploit these niches suggest that there is nothing inevitable about replication in macrophages. The key question then becomes what might be attractive about macrophages to a bacterial pathogen? If the choice of replicative niche, whether phagosomal, cytosolic, or extracellular, involves a set of trade-offs, what are the beneficial features of the macrophage niche that compensate for its more obviously detrimental antimicrobial properties (Figure 2)?

Diverse Metabolic Niches for Bacteria?

The idea that a high degree of metabolic diversity and plasticity makes macrophages attractive hosts for intracellular bacteria is an interesting lens through which to consider the macrophage paradox. The diversity of functions that macrophages perform throughout the body is underwritten by their ability to rapidly remodel their metabolism in response to specific environments

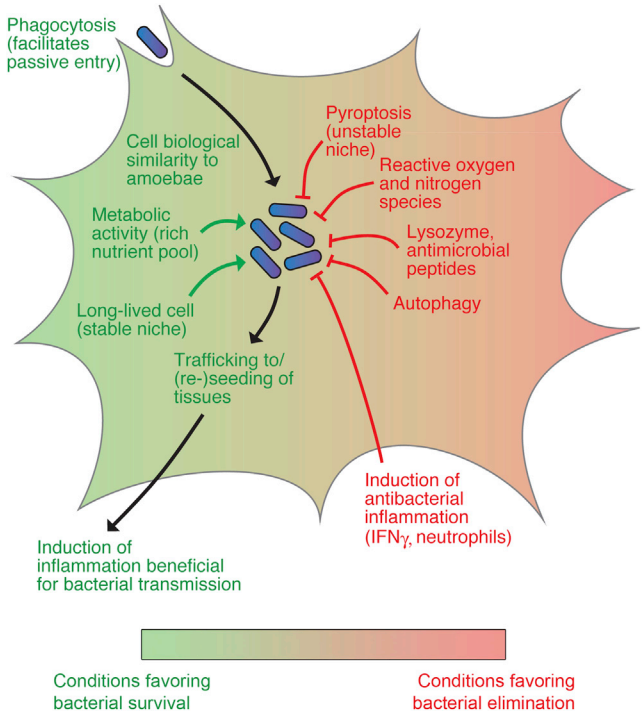

Figure 2. Resolutions of the Macrophage Paradox
Depicted are various factors that either favor (green) or disfavor (red) the macrophage as a niche for bacterial replication. Although macrophages encode numerous antimicrobial activities, the biology of macrophages is highly constrained by the diverse functions they play in tissues. We propose there may be many non-mutually-exclusive factors that, on balance, favor preferential bacterial replication in the macrophage niche.

and stimuli. During infection, discrete metabolic programs are engaged upon macrophage exposure to bacterial molecules and other external cues such as cytokines and immune complexes (Martinez and Gordon, 2014). To date, the study and understanding of macrophage metabolic plasticity during infection or inflammation has focused mainly on how metabolic shifts are tied mechanistically to specific macrophage immune functions (Ganeshan and Chawla, 2014; Ghesquière et al., 2014). Here we provide a general outline of these metabolic changes and subsequently focus on the question of how intracellular bacteria might take advantage of the diverse metabolic environments of macrophages to suit their own replication requirements.

Macrophages have traditionally been divided into two main subsets: "M1" or classically activated macrophages and "M2" or alternatively activated macrophages. Although this binary paradigm is clearly an oversimplification, and in reality macrophages encompass a spectrum of cellular activities and phenotypes (Martinez and Gordon, 2014; Murray et al., 2014), contrasting M1 and M2 activation states remains useful in our discussion of macrophage metabolism. M1 macrophages arise in response to IFN-γ, bacterial molecules such as lipopolysaccharide (LPS), or combinations of these stimuli, and are especially adept at bacterial killing. To fuel their energetic demands, M1 macrophages increase glucose uptake and glycolytic metabolism, which is tied to increased production of reactive oxygen species and the biosynthesis of cytokines (Ganeshan and Chawla, 2014). During enhanced glycolysis, which involves the reduction of NAD^+ to

NADH, increased activity of lactate dehydrogenase converts pyruvate into lactate to regenerate NAD^+ and prevents carbon from glucose from entering the tricarboxylic acid (TCA) cycle (Chiarugi et al., 2012; Ganeshan and Chawla, 2014). M1 macrophages generate TCA intermediates through increased uptake of glutamine, which is processed to α-ketoglutarate via glutaminolysis (Tannahill et al., 2013). Production of intracellular nitric oxide in LPS-stimulated cells have been shown to be toxic to mitochondria, resulting in further dependence on glycolysis in these cells (Everts et al., 2012). However, mitochondria also play a role in generating reactive oxygen species and in hosting the reactions of the TCA cycle, key metabolic underpinnings of the antimicrobial activities of M1 macrophages. In sum, the central carbon metabolism of M1 macrophages appears optimized for rapid biosynthesis of macromolecules and resembles aerobic glycolysis or the "Warburg effect" seen in some cancer cells (Tannahill et al., 2013; Vander Heiden et al., 2009). However, unlike the Warburg shift seen in tumor cells, the metabolic changes observed in M1 macrophages do not promote increased cell proliferation, but are instead thought to be critical to support the considerable biosynthetic demands encountered during the initiation of the immune response.

By contrast, macrophages polarized along the M2 activation spectrum are critical players in the Th2-cell-associated antiparasite response as well as in the resolution of inflammation and the promotion of tissue repair. Phenotypically distinct M2 macrophages arise in response to different stimuli, including Th2-cell-associated cytokines IL-4 and IL-13, bacterial molecules such as LPS in combination with immune complexes, and glucocorticoids, among others (Martinez and Gordon, 2014; Murray et al., 2014). M2 macrophages upregulate oxidative mitochondrial metabolic pathways (oxidative phosphorylation and fatty acid oxidation) and initiate mitochondrial biogenesis. Upregulation of fatty acid oxidation in IL-4- and/or IL-13-stimulated macrophages is dependent on STAT6 transcription factor signaling and activation of peroxisome proliferator-activated receptors (PPARs) PPARγ and PPARδ (Chawla, 2010). In comparison with the dramatic and more short-lived metabolic surges observed in M1 macrophages, the sustained oxidative metabolism of M2 cells might enable the extended immune campaigns necessary to eliminate parasites and the longer-term repair of tissues damaged during infection and inflammation. The overall view that emerges is that the macrophage polarization spectrum provides a corresponding variety of metabolic environments that metabolically diverse and adaptable microbes could exploit.

The M2 Macrophage Niche

To date, the evidence for M2 macrophages being more metabolically hospitable for intracellular parasitism has been mostly indirect. In several cases, it appears that a chronic inability to clear intracellular bacteria is associated with the elaboration of M2 macrophages. *Mycobacterium tuberculosis*, *Listeria monocytogenes*, and *Francisella tularensis* appear to induce M2 phenotypic characteristics in macrophages; however, the case of *F. tularensis* might be complicated by distinct activities of IL-4 during infection (Abdullah et al., 2012; Ketavarapu et al., 2008; Mahajan et al., 2012; Rajaram et al., 2010; Rodriguez et al., 2011). Although we still do not yet have a comprehensive understanding of the phenomena described in these studies, it appears that some intracellular bacteria can induce an M2 activation state in host macrophages and/or take up residence in M2 macrophages during infection.

The above studies invoke the reduced antimicrobial capacity of M2 macrophages to explain increased bacterial replication in these cells. However, two recent studies have identified the altered metabolic state of M2 macrophages as a further factor that supports the persistence of the intracellular pathogens *Salmonella enterica* serovar Typhimurium and *Brucella abortus* (Eisele et al., 2013; Xavier et al., 2013). In humans and mice, infection with *Salmonella* is associated with a robust immune response that requires the IL-12 and IFN-γ signaling axis for bacterial clearance (Jouanguy et al., 1999; Pie et al., 1997). However, some infected humans fail to completely clear the bacteria, resulting in chronic infections and the risk of transmission (Gopinath et al., 2012). Interestingly, Eisele et al. (2013) observed that *S.* Typhimurium bacteria are predominantly found in splenic M2 macrophages after oral infection. Their further experiments revealed that *S.* Typhimurium preferentially replicates in M2 cells and that the ability of *Salmonella* to exploit this niche required the host transcription factor PPARδ. Pharmacological or genetic inhibition of PPARδ diminished the ability of *Salmonella* to replicate, an effect that was tied to the decreased availability of intracellular glucose. The authors propose that increased macrophage oxidative metabolism, which is favored in M2 macrophages over the heavily glucose-fueled metabolism of M1 cells, allows *Salmonella* to capitalize on an increased amount of glucose for its own consumption. Furthermore, Eisele et al. (2013) demonstrated that infection of macrophages with *S.* Typhimurium induces PPARδ expression in macrophages, indicating that *Salmonella* might have the capacity to polarize its host macrophages to an M2 activation state, from which the bacteria can derive multilayered benefits.

Brucella abortus, another glucose-loving intracellular microbe, appears to similarly exploit an abundance of glucose in M2 macrophages, driven by PPARγ (Xavier et al., 2013). In this study, *B. abortus* survival and replication was increased in M2 cells and was dependent on the ability of the bacteria to access higher amounts of intracellular glucose measured in these macrophages. This study is in line with a previous investigation of the cytokine profile of human patients with chronic brucellosis, which linked prolonged disease duration with low IFN-γ and increased IL-13 (Rafiei et al., 2006). Notably, in contrast to *Salmonella* and the microbes discussed above, *Brucella* does not induce an M2 activation state in macrophages infected in vitro. In fact, *Brucella* induces an M1-type activation state in bone-marrow-derived macrophages infected in vitro, associated with upregulated glycolytic metabolism in these macrophages (Xavier et al., 2013). This indicates that although some intracellular bacteria might directly influence the polarization and metabolism of host cells to suit their metabolic needs, others, such as *Brucella*, could exploit a pre-existing diversity of macrophages to find a metabolically optimal niche for replication.

Although the existing literature supports the idea that some intracellular bacteria appear to prefer the M2 macrophage niche, an intriguing possibility is that some intracellular bacteria could exploit the potentially metabolite-rich environment within M1 macrophages, despite the enhanced antimicrobial activities of

these cells. The existence of a spectrum of macrophage activation states in vivo might allow some intracellular bacteria to find an optimal niche within a population of M1 cells, in which they avoid being killed while still reaping the metabolic reward of upregulated biosynthetic pathways in these cells. Increasingly sophisticated profiling strategies that are able to measure the contribution of host metabolites to intracellular bacterial replication during infection (Schunder et al., 2014) will help to further reveal the interplay between macrophage metabolic plasticity and intracellular bacterial replication. In order to assess whether metabolic remodeling provides an explanation for the propensity of macrophages to serve as host cells for bacterial pathogens, it will also be important to determine whether neutrophils or other cell types similarly remodel their metabolism in ways that are of potential benefit to microbial pathogens.

In recent years, genetic perturbation of key pathways involved in macrophage activation and metabolism has facilitated the study of mice with severely attenuated M1 or M2 activation states and deficiencies in entire macrophage subsets. For example, PPAR-deficient macrophages lack the ability to remodel their metabolism in response to M2 stimuli, and deficiencies in the IRF4 transcription factor pathway result in the complete absence of M2 macrophages arising from IL-4 stimulation (Chawla, 2010; Date et al., 2014; Satoh et al., 2010). In the studies of *Salmonella* and *Brucella* discussed above, PPAR-deficient mice were useful in elucidating the role of M2 macrophage metabolism in *Salmonella* and *Brucella* pathogenesis. It will be informative to make further use of the expanding number of genetic models affecting macrophage activation states to probe the extent to which intracellular bacteria capitalize on macrophage metabolic plasticity for optimal replication during infection in vivo.

Macrophages as an Amoebae-like Niche

Free-living single-celled phagocytic amoebae that feed on bacteria are ubiquitous in nature. These eukaryotic predators and their bacterial prey have been locked in an evolutionary struggle for millions if not billions of years (Hilbi et al., 2007). The fundamental cell biology of phagocytosis and phagosome maturation is largely conserved between amoebae and macrophages. In this light, an intriguing possibility is that, for some pathogens at least, macrophages are a familiar niche, not simply a hostile one.

An appreciable number of bacterial species exhibit the capacity to replicate or survive in amoebae (Greub and Raoult, 2004). Although there is not an extensive body of literature characterizing the explicit mechanistic interaction of most of these pathogens with amoebae, many species pathogenic to humans can infect amoebae, including *Coxiella*, *Burkholderia*, *Francisella*, *Listeria*, *Salmonella*, *Mycobacteria*, and *Shigella* (Brandl et al., 2005; Greub and Raoult, 2004; Huws et al., 2008; La Scola and Raoult, 2001; Saeed et al., 2009; Schuppler, 2014). *Legionella pneumophila* provides one particularly well-characterized example of how amoebae might provide a solution to the macrophage paradox. In humans, *Legionella* preferentially replicates in alveolar macrophages, but the natural host cells for *Legionella* are diverse freshwater amoebae (Fields, 1996). *Legionella* is considered an ''accidental pathogen'' of humans, because it is able to infect macrophages and cause severe pneumonia but has not evolved the ability to be transmitted between mammalian hosts. The advent of indoor water heating and cooling systems, in existence only for the most recent sliver of *Legionella*'s evolutionary history, has brought amoebae harboring *Legionella* into contact with human alveolar macrophages via inhaled aerosolized water droplets. Because the human host is a dead end for the bacteria, it is not likely that coevolution with mammalian macrophages influences the pathogenicity or virulence mechanisms of *Legionella* in the wild. Instead, adaptations that *Legionella* evolved to survive in a diverse group of free-living protozoan species have evidently granted it the ability to survive in macrophages as well. These adaptations include an arsenal of >300 secreted effector proteins that *Legionella* delivers to the host cytosol via a type IV secretion system (Hubber and Roy, 2010). *Legionella* encodes multiple effectors with overlapping and redundant activities, presumably equipping it to cope with the diversity of its amoebal hosts (O'Connor et al., 2011). Macrophages, then, are just another environmental phagocyte from the perspective of *Legionella*, whose effector arsenal is sufficiently broad to permit parasitization of macrophages. Indeed, where it has been dissected, the host mechanisms targeted and/or exploited by *Legionella* in amoebae and macrophages tend to be identical (Molmeret et al., 2005; Segal and Shuman, 1999).

These observations point to a model in which some intracellular bacteria, evolved to resist predation by free-living amoebae, are able to parasitize human cells when circumstances such as new human technologies inadvertently bring amoebae, their intracellular bacterial cargo, and humans into proximity. Highly conserved host targets of bacterial virulence factors and functional similarity between free-living amoebae and macrophages (i.e., a high phagocytic capacity, conserved endocytic and metabolic machinery) might allow intracellular bacteria to transition from protozoan to mammalian host cells with relative ease. In fact, *Legionella* passaged for hundreds of generations in macrophages not only increased their ability to replicate in macrophages, but lost the ability to efficiently replicate in cultured amoebae, changes tied to flagellar regulation and the advent of lysine auxotrophy (Ensminger et al., 2012). This experiment provides tantalizing evidence for the notion that bacteria with the ability to infect amoebae can adapt to become mammalian pathogens by taking advantage of their ability to replicate in macrophages. In this light, the seemingly high number of intracellular bacteria that ''prefer'' to replicate in macrophages might in fact reflect the role of the macrophage as a ''gateway'' mammalian cell for bacteria with preexisting tools for replication in amoebae.

Other Resolutions to the Macrophage Paradox

We favor the view that there are probably many mutually nonexclusive reasons to explain why intracellular bacterial pathogens would favor the macrophage niche. Moreover, it is likely that the reasons are not necessarily the same for all pathogens. One intriguing idea that might apply to certain pathogens is that parasitization of macrophages provides a mechanism for a pathogen to spread to systemic sites within its host (Vazquez-Torres et al., 1999). However, the extent to which infected macrophages circulate among tissues is not well established. Moreover, for a pathogen such as *Salmonella*, which is typically transmitted via the fecal-oral route, the benefit of spreading deep into systemic tissue is unclear, if indeed it is the intestinal luminal bacteria that are ultimately transmitted to the next host. One

attractive idea is that infected macrophages might serve as an alternative reservoir of bacteria from which the gut lumen can be reseeded. A related idea is that infection of macrophages might induce an inflammatory environment that benefits the pathogen indirectly. For example, intestinal luminal *S.* Typhimurium are able to compete metabolically with the host microbiota by feeding off electron acceptors, such as tetrathionate, that are produced as a consequence of gut inflammation (Winter et al., 2010). *Salmonella* bacteria that infect lamina propria macrophages might themselves be killed, but might nevertheless assist transmission of their luminal brethren by helping to provoke a beneficial inflammatory state.

Interaction of Other Pathogenic Microbes with Macrophages

The apparent preference of bacteria for replication in macrophages can be contrasted with the cellular niches preferred by viruses, fungi, and protozoan parasites. Although a number of microbes in each of these categories do infect macrophages, there does not appear to be a similar propensity for these organisms to single out macrophages above others as host cells (Mercer and Greber, 2013; Sibley, 2011). The reasons for the distinct cellular preferences of bacteria and other pathogens are not clear. As discussed above, macrophages are able to generate a high amount of type I IFNs, which are almost universally effective against viruses, but exhibit much more variable effects on bacteria (Monroe et al., 2010). Viruses also rely on host translation for their replication, which makes them particularly susceptible to host-mediated inhibition of host protein synthesis (Mohr and Sonenberg, 2012). Conversely, because pathogenic intracellular bacteria possess their own biosynthetic machinery, they might be more interested in accessing stores of host metabolites and less concerned about protein synthesis inhibition. Some bacteria even possess their own mechanisms for the inhibition of protein synthesis, as in the case of *Legionella*, *Shigella*, and *Pseudomonas* (Belyi et al., 2008; Sandvig and van Deurs, 1996; Wilson and Collier, 1992). Additionally, macrophages express the deoxynucleotide triphosphate (dNTP) hydrolase SAMHD1, which restricts retrovirus pathogenesis through limiting the availability of cytosolic dNTPs for viral genome replication (Ayinde et al., 2012). As discussed previously, given the extraordinary adaptability of pathogens, the presence of multiple defense strategies does not per se render a cell inhospitable to a particular class of organism, and indeed, many viruses have evolved mechanisms to subvert the cell-autonomous and non-cell-autonomous immune defenses of macrophages and other host cells. However, as immune sentinels, macrophages' tool kit might be better optimized to combat viruses than intracellular bacteria.

The pathogenic fungi *Histoplasma capsulatum* and *Cryptococcus neoformans* have the ability to replicate in the phagosomes of phagocytic cells, including macrophages; however, this trait does not appear to be common among fungal pathogens (Feldmesser et al., 2001). Although some protozoan parasites, including *Toxoplasma gondii*, *Trypanosoma cruzi*, and *Leishmania spp.* (Bogdan and Röllinghoff, 1999), take up residence and even replicate within macrophages, overall it appears that parasite intracellular replication in macrophages is rare (Sibley, 2011). Of the species that do replicate in macrophages, only *Leishmania* appears to be specialized for replication in macrophages above other cell types (Stafford et al., 2002).

In sum, within the spectrum of commonly studied microbial pathogens, it appears that intracellular bacteria might be particularly poised to exploit macrophages for replication. Given the evolutionary pressures imposed upon macrophages as first responders to infection by highly diverse pathogens, it is perhaps not surprising that they are rendered differentially susceptible to viral, fungal, protozoan, and bacterial pathogens. It should be noted that our knowledge of the preferred mammalian cell types for many pathogens, including bacteria, has been greatly influenced by studies using in-vitro-derived cells or primary cells cultured ex vivo. Data for the preferred host cells in vivo remain lacking for many pathogens. Live tracking of microbes and host cells in vivo enabled by advances in cell labeling and detection technologies will provide definitive evidence of pathogens' biases toward subsets of macrophages and other specific cellular niches.

Conclusion: Why Does the Macrophage Paradox Matter?

We have noted the surprising extent to which intracellular bacterial pathogens exploit macrophages as an intracellular niche despite macrophages' well-characterized antimicrobial activities. We have also speculated that the special "paradoxical" relationship between bacteria and macrophages probably arises from many facets of the complex biology of macrophages. However, we have not yet made an argument for why consideration of the macrophage paradox is important. In fact, we believe that the macrophage paradox is central to understanding the immunology and microbiology of intracellular bacterial pathogens. The macrophage paradox forces one to consider and weigh the constraints faced by intracellular pathogens and their host cells. Without addressing the macrophage paradox, it is not possible to really understand why so many bacterial pathogens devote considerable genetic and energetic resources to produce dedicated secretion systems and other virulence factors that (largely) serve to suppress or escape macrophage defenses. From the host's perspective, once faced with the macrophage paradox, it is no longer possible to think of macrophages as simple antimicrobial effector cells. Instead, macrophages must be envisioned as active and highly environmentally responsive cells that exhibit—and therefore provide to pathogens—a diversity of metabolic and cellular states. Understanding both the appeal and the limitations of the macrophage niche helps us understand the challenges and opportunities faced by newly emerging pathogens as they try to exploit macrophages, as so many established pathogens have already done before them. Ultimately, our hope is that articulation of the macrophage paradox will lead to a better understanding of what makes macrophages attractive or vulnerable hosts for bacterial pathogens, which might then facilitate the design of host-directed therapeutic interventions that limit macrophages' attractiveness or vulnerability.

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