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Microreview

Microbial quest for food *in vivo*: 'Nutritional virulence' as an emerging paradigm

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Summary

Microbial access to host nutrients is a fundamental aspect of infectious diseases. Pathogens face complex dynamic nutritional host microenvironments that change with increasing inflammation and local hypoxia. Since the host can actively limit microbial access to nutrient supply, pathogens have evolved various metabolic adaptations to successfully exploit available host nutrients for proliferation. Recent studies have unraveled an emerging paradigm that we propose to designate as 'nutritional virulence'. This paradigm is based on specific virulence mechanisms that target major host biosynthetic and degradation pathways (proteasomes, autophagy and lysosomes) or nutrient-rich sources, such as glutathione, to enhance host supply of limiting nutrients, such as cysteine. Although Cys is the most limiting cellular amino acid, it is a metabolically favourable source of carbon and energy for various pathogens that are auxotrophic for Cys but utilize idiosyncratic nutritional virulence strategies to generate a gratuitous supply of host Cys. Therefore, proliferation of some intracellular pathogens is restricted by a host nutritional rheostat regulated by certain limiting amino acids, and pathogens have evolved idiosyncratic strategies to short circuit the host nutritional rheostat. Deciphering mechanisms of microbial 'nutritional virulence' and metabolism *in vivo* will facilitate identification of novel microbial and host targets for treatment and prevention of infectious diseases. Host–pathogen synchronization of amino acid auxotrophy indicates that this nutritional synchronization has been a major driving force in the evolution of many intracellular bacterial pathogens.

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Introduction

Over the past two decades, our understanding of the molecular and cellular aspects of microbial pathogenesis has witnessed a quantum leap. This has been fuelled by the discovery of type III, IV, VI and VII translocation systems that inject a large cadre of eukaryotic-like and novel microbial effectors into the host cell. These effectors manipulate a myriad of host cell processes and subvert innate and adaptive immunity through novel and exciting mechanisms. New evidence shows that some of the microbial injected effectors are dedicated to extracting nutrients needed for pathogen proliferation in the host. This review discusses recent advances in our knowledge of unique microbial nutritional adaptation to the host and novel microbial mechanisms dedicated to extract sufficient host nutrients for pathogen proliferation in infected tissues. For other aspects of microbial nutrition *in vivo*, the reader may refer to other recent reviews (Raffatellu *et al.*, 2009; Eisenreich *et al.*, 2010; Rohmer *et al.*, 2011; Bliven and Maurelli, 2012; Fuchs *et al.*, 2012).

Microbial nutritional adaptation *in vivo* as a prerequisite for infectious diseases

Nutrition and proliferation is fundamental to life, and all organisms have evolved to maximize their harvest of energy and biomass building blocks from available nutrients. This applies also to pathogens that colonize host tissues and cause disease. Indeed, without proper nutritional resources for survival/proliferation in the host, bacterial pathogens do not cause disease.

It can be challenging for microbial pathogens to obtain nutrients during infection, since part of the host innate

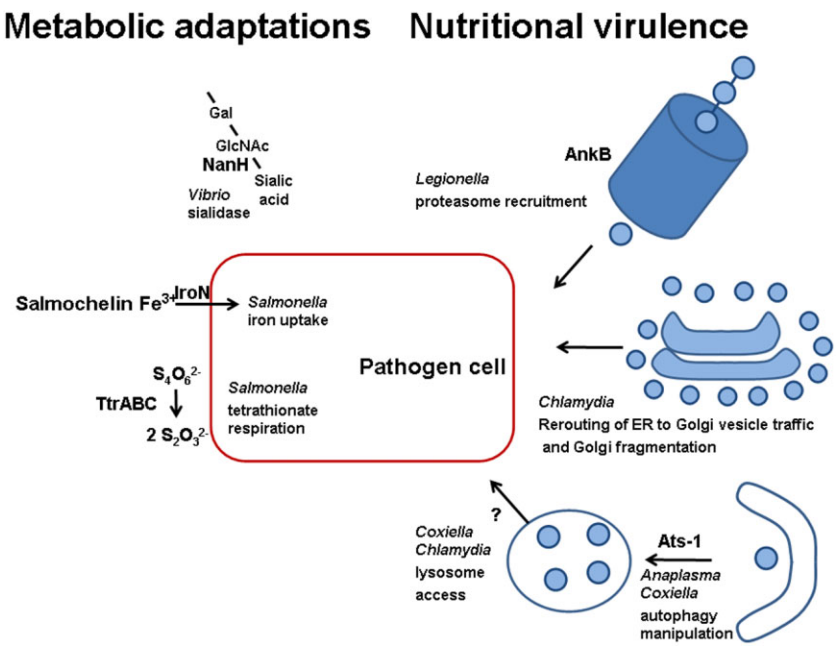

Fig. 1. Nutrition acquisition strategies of microbial pathogens in infected host tissues. Common microbial metabolic adaptations are shown on the left side (pathogen factors are shown in red). Pathogen 'nutritional virulence' strategies that target host biosynthetic and degradation machineries by virulence factors to tap into host-derived nutrients are shown on the right side. *Coxiella* or *Chlamydia* factors facilitating lysosome access have not yet been identified.

defence is to restrict pathogen access to various essential nutrients such as iron. Successful pathogens have therefore evolved highly efficient nutrient retrieval strategies to counteract this nutritional deprivation by the host (Winter *et al*., 2010; Price *et al*., 2011; Lopez *et al*., 2012; Niu *et al*., 2012; Steeb *et al*., 2013). As an example, the *iroBCDE* gene cluster of *Salmonella* is dedicated to the biosynthesis and uptake of the iron-binding siderophore, salmochelin, that is resistant to degradation by the host lipocalin-2, which inactivates other bacterial siderophores during inflammation, giving *Salmonella* a competitive edge over the microbiota because of better access to essential iron (Fig. 1) (Raffatellu *et al*., 2009). Some pathogens such as *Shigella* and *Listeria* have also evolved the ability to grow in the host cell cytosol where they have direct access to rich nutrients.

Microbial nutrition during infection is also challenging since pathogens usually encounter several different host niches/tissues with unique nutrient availability. Even within the same niche, nutrient supply might be highly dynamic due to the immune response and the associated inflammation and hypoxic conditions, as well as degradation of macromolecules within inflammatory foci (Nizet and Johnson, 2009). Tissue hypoxia triggers metabolic modulation in host cells with a dramatic shift to upregulate transcription of glycolytic enzymes and the glycolysis pathway, leading to generation of high local levels of lactic acids and increased local acidity (Nizet and Johnson, 2009). As an example, persistence of *Mycobacterium tuberculosis* in the granuloma is dependent on utilization of fatty acids and cholesterol as a carbon source within the inflamed granuloma (McKinney *et al*., 2000; Thi *et al*., 2012). Thus, pathogens have evolved unique metabolic adaptations to various complex and dynamic *in vivo* nutritional microenvironments to maximize nutrient harvest from host resources.

Although microbial acquisition of nutrients and metabolism *in vivo* is a major fundamental aspect of infectious diseases impacting on virulence, pathology and efficacy of antibiotic treatment (Nguyen *et al*., 2011; Rohmer *et al*., 2011), our knowledge of microbial metabolism *in vivo* still remains very limited. This is likely due to the major focus on 'classical virulence', novel microbial pathogenic factors, as well as the experimental challenges to decipher microbial nutrition and metabolism within complex microenvironments in the host. Moreover, many metabolic capabilities are mediated by redundant enzymes, and these can easily escape identification by conventional genetic approaches. On the other hand, numerous global genome-wide transcriptome and mutant screens have identified a large cadre of metabolic genes and pathways that are expressed *in vivo*. In most cases, underlying mechanisms and their functional relevance remain unclear, but extensive current research continuously provides new information that might ultimately result in an integrated understanding of pathogen nutrition and metabolism *in vivo*. Such data will also provide the required foundation for microbial genome-scale metabolic

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reconstructions to systematically identify drug targets (Chavali *et al*., 2012).

Genetic acquisition of novel metabolic pathways facilitates pathogen nutrition

Several bacterial pathogens meet the challenging nutritional requirements during infection by acquiring genes from other microbes through horizontal transfer of 'pathogenicity islands' and other mobile elements (Prunier *et al*., 2007; Yus *et al*., 2009; Rohmer *et al*., 2011), similar to what has been extensively documented for 'classical' virulence genes. The acquired genes enable specific metabolic conversions, or code for bacterial effectors injected into the host to manipulate nutrient supply from host sources or enzymes to degrade host macromolecules (Alkhuder *et al*., 2009; Winter *et al*., 2010; Price *et al*., 2011; Lopez *et al*., 2012; Niu *et al*., 2012).

As an example, *Salmonella enterica* serovar Typhimurium uses horizontally acquired genes for anaerobic respiration in the inflamed intestine. Specifically, the *ttrABCRS* gene cluster encoded within the *Salmonella* pathogenicity island 2 enables the microbe to utilize tetrathionate as an electron acceptor for respiration in the intestine (Winter *et al*., 2010). Interestingly, *Salmonella* anaerobic respiration is not static but continuously shaped by ongoing evolution. In fact, some *Salmonella* clones have additionally acquired a lysogenic phage that also encodes the translocated effector SopE triggering enhanced production of host-derived nitrate (Lopez *et al*., 2012). Within the anaerobic microenvironment in the inflamed intestine nitrate is actually an energetically favourable electron acceptor compared with tetrathionate. Thus, acquisition of *sopE* allows anaerobic nitrate respiration and suppression of genes dedicated to utilization of the inferior acceptor tetrathionate (Fig. 1) (Lopez *et al*., 2012). Closely related *S. enterica* serovars Typhi and Paratyphi that primarily proliferate in systemic tissues where tetrathionate is unavailable, have even lost the *ttrABCRS* gene cluster (Winter *et al*., 2010). Recent studies have also shown that host-derived nitrate generation in the inflamed intestine of mice confers a growth advantage to commensal *E. coli* by generating an electron acceptor for anaerobic respiration (Winter *et al*., 2013). Thus, to adapt to specific nutritional and dynamic microenvironments in infected and inflamed host tissues, pathogens have lost genes (Bliven and Maurelli, 2012) or horizontally acquired new genes dedicated to maximize exploitation and extraction of nutrients from the host in specific niches.

Pathogen nutrient retrieval by triggering major host protein degradation pathways

Degradation of host macromolecules by specific microbial enzymes, such as proteases, lipases and phospholipases, is a very efficient and direct strategy to extract nutrients from the host. For example, a neuraminidase encoded within the VPI2 pathogenicity island of pathogenic strains of *Vibrio cholerae* hydrolyses host sialic acid to generate carbon sources and to enable toxin binding (Fig. 1) (Almagro-Moreno and Boyd, 2010). In contrast to such microbial degradation, other pathogens exploit host degradation processes to mobilize and access important host nutrients such as amino acids needed for pathogen proliferation (Fig. 1).

Lysosomes are the major vesicle that contains host cellular degradation enzymes, and this degradation compartment provides a source of amino acids necessary for intracellular growth of some pathogens such as *Coxiella* and *Chlamydia* (Fig. 1) (Ouellette *et al*., 2011). It is also possible that the host endoplasmic reticulum associated degradation (ERAD) machinery can be targeted by pathogens to enhance nutrient supply but this has not yet been conclusively demonstrated. However, recent studies have provided new paradigms for pathogen exploitation of host major degradation pathways, such as proteasomal degradation and autophagy, to meet the pathogen's demand for amino acids as metabolically favourable sources for carbon and energy to power robust intracellular bacterial proliferation (Price *et al*., 2011; Niu *et al*., 2012).

Proteasomes are protein degradation machineries present in all eukaryotic organisms as well as *Archaea* (Gallastegui and Groll, 2010) and *M. tuberculosis* (Cerda-Maira and Darwin, 2009). They degrade abnormal and misfolded proteins tagged with poly-ubiquitin or related tags (Fig. 2) (Gallastegui and Groll, 2010). On the other hand, autophagy is a highly regulated eukaryotic cellular homeostatic process that sequesters and digests damaged organelles and intracellular components through engulfment by membranes to form an autophagosome that fuses to the lysosomes (Deretic, 2012; Kuballa *et al*., 2012). Autophagy plays important roles in immunological processes, including direct pathogen elimination, pathogen-associated molecular pattern processing for pattern recognition receptor, inflammasome regulation and autosecretion of alarmins, and cytosolic antigen processing for MHC-II presentation (Deretic, 2012; Kuballa *et al*., 2012). Autophagy is thus one of the primary innate immune defence mechanisms against invading viruses, bacteria and protozoa (Deretic, 2012; Kuballa *et al*., 2012).

L. pneumophila satisfies its appetite for amino acids through promoting host proteasomal degradation

Legionella pneumophila is an environmental organism that proliferates within amoeba in the aquatic environment (Al-Quadan *et al*., 2012). Upon transmission to humans through aerosols, *L. pneumophila* infects

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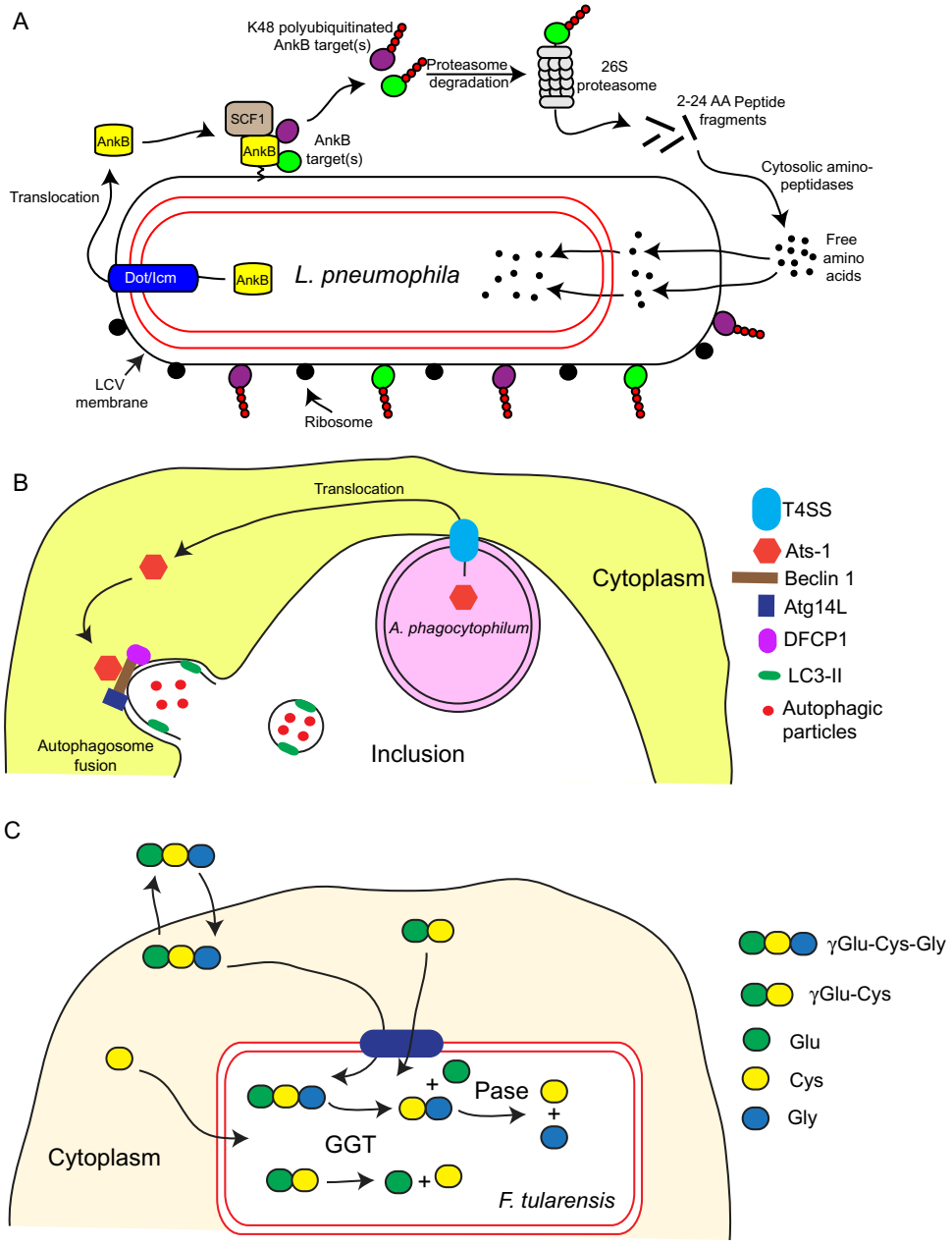

Fig. 2. Nutritional virulence of *Anaplasma*, *Legionella* and *Francisella* to trigger host cells to generate a gratuitous levels of metabolically favourable amino acids that are limiting in the host cell is essential for intracellular bacterial proliferation.
A. The Dot/Icm type IV secretion-translocated AnkB effector of *L. pneumophila* is farnesylated by the host enzymes to enable its exclusive anchoring into the cytosolic face of the PCV membrane where it interacts with the eukaryotic ubiquitin ligase complex and serves as a platform for the docking of K^{48}-linked poly-ubiquitinated proteins. Proteasomal degradation of the poly-ubiquitinated protein generates peptides and then amino acids that are imported into the PCV.
B. The type IV secretion-translocated Ats-1 effector of *Anaplasma* is required for Atg14L recruiting and thus, autophagosome formation. The outer membrane of the autophagosome, which is decorated with LC3, fuses with the PCV membrane, resulting in the release of the autophagic body-like content into the PCV (designated inclusion) lumen.
C. Utilization of GSH and γ-Glu–Cys by cytosolic *F. tularensis*, which can utilize GSH (and its oxidized form GSSG), γ-Glu–Cys, cysteine, and cystine as sources of cysteine. In host cells, cytosolic GSH, the most abundant thiol-containing compound, and γ-Glu–Cys are processed by GGT to produce γ-Glu and Cys–Gly or γ-Glu and Cys respectively. The Cys–Gly dipeptide is further processed by other amino-acid peptidases to produce free cysteine and glycine. The available cellular concentration of free cysteine is limiting and not sufficient to promote intracellular growth of *Legionella* and *Francisella* that utilize unique strategies to trigger the host cell to generate gratuitous levels of this limiting amino acid. Whether generation of amino acids through promoting autophagy by *Anaplasma* is mainly intended to raise the levels of cellular Cys or other essential amino acids is more likely, but remains to be determined.

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alveolar macrophages where the bacteria proliferate and cause atypical pneumonia, designated as Legionnaires' disease. Within both macrophages and amoeba, *L. pneumophila* grows in a pathogen-containing vacuole (PCV) that evades lysosomal fusion and is remodelled by the endoplasmic reticulum (Al-Quadan *et al*., 2012). Amino acids that are metabolized primarily through the tri-carboxylic acid (TCA) cycle, represent the main sources of carbon and energy for *L. pneumophila*, which does not have a functional glycolytic pathway. Interestingly, the organism has an absolute requirement for external high levels of cysteine, which feeds into the TCA cycle after its conversion to pyruvate, as a metabolically favourable source of carbon and energy. *L. pneumophila* injects into the host cells an eukaryotic-like F-box protein effector, designated Ankyrin B (AnkB), which is lipidated by the host farnesylation machinery (Al-Quadan *et al*., 2011) to allow its anchoring to the lipid bi-layer of the PCV membrane (Fig. 2) (Price *et al*., 2010). On the PCV membrane, the AnkB effector functions as a platform for the assembly of Lys^{48}-linked poly-ubiquitinated proteins, which are targeted for degradation by the host proteasomes (Fig. 2) (Price *et al*., 2011). This generates elevated levels of cellular amino acids, which are imported into the PCV to sustain the restricted 'Aitkins' diet lifestyle of *Legionella* (Al-Quadan *et al*., 2012). Interference with the host Lys^{48}-linked poly-ubiquitination, inhibition of the host proteasomes, or ablation of the AnkB effector renders *Legionella* unable to grow *in vivo*, but this growth defect can be totally bypassed upon excess supplementation of a mixture of amino acids or by cysteine (Price *et al*., 2011).

Remarkably, as an alternatives to cysteine, pyruvate and citrate are both sufficient to rescue the *ankB* null mutant for its proliferation in amoeba and human macrophages, consistent with metabolism of cysteine into pyruvate and TCA cycle intermediates (Price *et al*., 2011). Since macrophages in hypoxic inflammatory foci undergo metabolic shift to anaerobic glycolysis (Nizet and Johnson, 2009), generation of high levels of host pyruvate under these conditions may boost intracellular growth of *L. pneumophila*, in addition to cysteine derived from promoting host proteasomal degradation.

It is thought that *ankB* has been acquired by *Legionella* through inter-kingdom horizontal gene transfer from the amoeba environmental host (Al-Quadan *et al*., 2012). As another result of nutritional patho-adaptation to the intracellular life within amoeba and human cells, *Legionella* has lost some of its own amino acid biosynthetic pathways (Leu, Ile, Met, Val, Thr, Cys). Remarkably, this gene loss has resulted in a synchronized amino acids auxotrophy with the two evolutionarily distant hosts (amoeba and humans), with the exception of cysteine, which is essential for amoeba but is semi-essential and the most limiting amino acids in humans (Price *et al*., 2011; Al-Quadan *et al*., 2012).

Promoting host autophagy for tapping amino acids supply for *A. phagocytophilum in vivo*

In addition to its role in recycling damaged cell components, autophagy is an innate immune defence against pathogen invasion, and many pathogens have evolved to evade this pathway thereby avoiding host degradation (Kuballa *et al*., 2012). However, other pathogens even trigger this degradation pathway, and these include *Anaplasma*, *Coxiella*, *Rickettsia*, *Helicobacter*, *Porphyromonas*, *Legionella* and *Brucella* (Kuballa *et al*., 2012) (Gutierrez and Colombo, 2005; Starr *et al*., 2012). This raises the questions whether microbial strategies to promote host autophagy degradation machinery might have been a pathogen strategy to improve nutrient supply. Indeed, recent studies on *Anaplasma phagocytophilum* show that this organism promotes autophagy to obtain amino acids essential for pathogen proliferation (Niu *et al*., 2012).

Anaplasma phagocytophilum, which belongs to the order *Rickettsiales*, is an obligate intracellular bacterium that infects granulocytes and endothelial cells of various mammalian species (Niu *et al*., 2008). In humans, *A. phagocytophilum* causes an emerging tick-borne disease called human granulocytic anaplasmosis, an acute febrile disease that is potentially fatal (Niu *et al*., 2008). The organism replicates inside the PCV that resembles an early autophagosome, but does not fuse to lysosomes (Niu *et al*., 2008). Inhibition of autophagy by the class III PI3K (PI3KC3) inhibitor 3-methyladenine (3-MA) arrests intracellular proliferation of *A. phagocytophilum*, while induction of autophagy significantly promotes its replication (Niu *et al*., 2008). The type IV secretion-translocated substrate 1 (Ats-1) effector of *A. phagocytophilum* binds Beclin 1, a subunit of the class III PI3K, and Atg14L, which become localized to the PCV (Fig. 2) (Niu *et al*., 2012). Ectopically expressed Ats-1 targets the PCV and enhances infection, whereas cytosolic neutralization of Ats-1 by a specific antibody or Beclin 1 knockdown by siRNA suppresses intracellular proliferation of *A. phagocytophilum*. In addition, mice that are heterozygous for Beclin 1 are resistant to *Anaplasma* infection suggesting strong dependency on normal levels of this host factor (Niu *et al*., 2012). Similar to rescue of proliferation arrest of *L. pneumophila* by amino acids in proteasome-inhibited cells (Price *et al*., 2011), intracellular growth arrest of *Anaplasma* in autophagy-inhibited cells is also restored by excess supplementation with essential amino acids (Niu *et al*., 2012) suggesting a primarily nutritional basis for microbial triggering of host autophagy for supporting *Anaplasma* growth *in vivo.*

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Interestingly, *Anaplasma* surface associated proteases are required for the degradation of proteins delivered to the PCV by autophagy, providing further support for the role of host autophagy as a source for microbial nutrition (Fig. 2) (Niu *et al*., 2012). Together, this illustrates an integrated microbial strategy that combines triggering host autophagy to deliver proteins into the PCV followed by the degradation of host proteins by bacterial proteases to satisfy microbial appetite for amino acids to support robust intracellular proliferation of the pathogen. As an obligate intracellular pathogen with a small genome, *A. phagocytophilum* is auxotrophic for most amino acids. It would be interesting to decipher which limiting cellular amino acids are needed at higher levels to support intracellular proliferation of *A. phagocytophilum.* Considering that this organism is auxotrophic for Cys, it is likely Cys is the limiting nutritional source for this organism *in vivo*.

It is likely that other pathogens, such as *Rickettsia*, *Coxiella* and *Brucella* (Gutierrez and Colombo, 2005; Kuballa *et al*., 2012; Starr *et al*., 2012) that trigger host autophagy to promote pathogen proliferation, also depend on this process to supply sufficient amino acids and/or other breakdown products to sustain the robust intracellular growth. This can be easily tested as has been already shown for *A. phagocytophilum* through the supplementation of amino acids to bypass the requirement of host autophagy for intracellular bacterial growth (Niu *et al*., 2012).

Together, the two examples of *L. pneumophila* and *A. phagocytophilum* (Fig. 2) provide direct links between microbial nutrition *in vivo* and virulence mediated by a virulence effector dedicated to promote major host degradation pathways to overcome host limitation of metabolically favourable sources of carbon and energy to support intracellular proliferation. Interestingly, other invasive intracellular pathogens such as *Shigella* and *Salmonella* trigger membrane damage-dependent host autophagy through eliciting an amino acid starvation response in the host cell as a defence mechanism (Tattoli *et al*., 2012).

Do limiting cellular amino acids function as a nutritional rheostat for intracellular bacterial proliferation?

Human cells are auxotrophic for phenylalanine, valine, threonine, tryptophan, isoleucine, methionine, leucine, lysine and histidine, while cystein is the most limiting amino acid (Young, 1994). Interestingly, recent studies have shown that eukaryotic cell death following extended inhibition of proteasomal protein degradation is due to depletion of cellular amino acids needed for protein synthesis, and cell death is totally blocked by supplementation of cysteine, which is the least abundant and most limiting cellular amino acid (Suraweera *et al*., 2012).

Various intracellular pathogens utilize certain amino acids as metabolically favourable sources of carbon and energy. However, the basal cellular levels of these preferred amino acids are not sufficient to support intracellular proliferation of many pathogens, including *L. pneumophila*, *A. phagocytophilum* and *F. tularensis.* Therefore, we propose that levels of host limiting cellular amino acids (Cys or essential amino acids) might be a nutritional rheostat that controls pathogen proliferation. Levels of available cysteine might be further limited by conversion to the dipeptide cystine, and many pathogens indeed express cystine transporters.

To short-circuit the host nutritional rheostat and boost cellular levels of metabolically favourable but limiting amino acids, pathogens exploit idiosyncratic strategies. *L. pneumophila* is auxotrophic for Cys and has evolved to exploit host proteasomal degradation to boost the cellular levels of Cys, which is a favourable source of carbon and energy needed for intracellular proliferation of *L. pneumophila* (Price *et al*., 2011). In contrast, *A. phagocytophilum*, which also is auxotrophic for Cys, triggers host autophagy as a source of limiting amino acids in the PCV (Niu *et al*., 2012).

Cysteine is also an essential amino acid for the cytosolic pathogen *Francisella tularensis* (Meibom and Charbit, 2010). In contrast to *L. pneumophila* and *A. phagocytophilum*, *F. tularensis* predominantly exploits host glutathione (GSH) (Alkhuder *et al*., 2009; Meibom and Charbit, 2010), which is the most abundant source of Cys in the host cytosol (Franco *et al*., 2007). Glutathione is a non-ribosomal tri-peptide (L-γ-L-glutamyl-L-cysteinyl-glycine) present in almost all eukaryotic cells and some prokaryotes, and its synthesis in eukaryotes is limited by the relatively low levels of cellular Cys (0.10–0.25 mM) (Franco *et al*., 2007). *F. tularensis* exploits GSH as a rich source of Cys by secreting the enzyme γ-glutamyl transpeptidase (GGT), which cleaves GSH to liberate Cys, raising the cellular levels of Cys needed to power intracellular proliferation in the host cell cytosol (Fig. 2) (Alkhuder *et al*., 2009; Meibom and Charbit, 2010). Similar to rescue of the intracellular growth defect of the *ankB* mutant of *L. pneumophila* by amino acids or by Cys and of *A. phagocytophilum* after blockage of Ats-1 by amino acids, the severe intracellular growth defect of the *ggt* mutant of *F. tularensis* is rescued by supplementation of Cys (Alkhuder *et al*., 2009). In addition, requirement for high levels of Cys for *in vitro* growth of *F. tularensis* is bypassed by supplementation of GSH (Alkhuder *et al*., 2009). Thus, the host nutritional rheostat regulated by limited Cys levels is short-circuited by *F. tularensis* through microbial targeting of the host glutathione, which is the richest source of Cys in the host cell.

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Therefore, at least two documented intracellular pathogens; one that proliferates within the PCV (*L. pneumophila*) and one that proliferates in the host cytosol (*F. tularensis*) are totally dependent on exploiting distinct cellular sources to generate high levels of cellular Cys, which is a metabolically favourable source of carbon an energy and is essential for intracellular proliferation of the two pathogens (Alkhuder *et al*., 2009; Price *et al*., 2011). *A. phagocytophilum* also exploits host autophagy to obtain an additional surplus of amino acids from the host to proliferate. It is most likely that other pathogens are also dependent on rich cellular sources to generate higher levels of certain limiting cellular amino acids (Cys and host essential amino acids) to support the high demand of bacterial proliferation (Niu *et al*., 2012). Remarkably, many pathogens have evolved to synchronize their amino acids auxotrophy with that of the host, which likely ensures availability of host nutrient supplies to power intracellular bacterial proliferation. Otherwise, microbial proliferation in a nutrient-deprived host is thought to be counter selective against the pathogen.

Microbial interception of host biosynthetic pathways to tap sources of nutrients

In addition to manipulation of cellular degradation processes to obtain nutrients, some pathogens also manipulate and intercept intracellular traffic of host metabolites with potentially important consequences for nutrient supply. As an example, *Chlamydia* is an intracellular bacterium that proliferates within a specialized PCV, which is excluded from the endocytic pathway. The type III secretion system of *Chlamydia* translocates the effector IncD that targets the PCV membrane and intercepts ER-to-Golgi vesicle traffic of ceramide, a precursor of sphingomyelin, which is essential for *Chlamydia* growth (Elwell *et al*., 2011). Furthermore, *Chlamydia* triggers fragmentation of the Golgi apparatus leading to enhanced nutrient delivery to *Chlamydia* (Christian *et al*., 2011). It is most likely that other intracellular microbial pathogens similarly target various host biosynthetic/trafficking pathways to tap nutrients.

Conclusions and future directions

Pathogen exploitation of host nutrients is a major battlefield for host–pathogen interactions, and is one of the most fundamental aspects of infectious diseases. In addition to classical metabolic adaptations that are generally used by microbes to efficiently exploit available nutrients in diverse environments, pathogens have also evolved specific virulence mechanisms that directly manipulate host nutrient supply. We propose to designate the term 'nutritional virulence' to define such unique pathogen nutritional strategies. By this terminology, AnkB of *L. pneumophila*, *ggt* of *F. tularensis*, and Ats-1 of *Anaplasma* can be termed as 'nutritional virulence factors'. It is likely that many classical pathogen virulence mechanisms that result in manipulation of host cellular processes, inflammatory responses, and tissue damage may have an additional, yet unrecognized nutritional component. As an example, tissue damage by various microbial hydrolytic enzymes, such as proteases and lipases, is likely to generate suitable microbial sources of carbon/nitrogen/sulfur and energy, but the functional relevance of this for pathogen survival/growth is still unclear. Deciphering these interconnections will require integrated approaches that combine classical methods of cellular microbiology, *in vivo* metabolomics, and metabolic pathway analyses to provide detailed knowledge of pathogen nutrient acquisition and metabolism *in vivo*.

Such integrated knowledge has already enabled major breakthroughs such as axenic *in vitro* cultivation of *Coxiella* and metabolic and biosynthetic activities of *Chlamydia*, which had both previously been considered obligate intracellular pathogens (Omsland *et al*., 2009; 2012), thereby opening up entirely new opportunities for research on these important pathogens. Similar seminal discoveries might be possible for other still 'obligate' intracellular pathogens such as *Rickettsia* and *Anaplasma*. In addition to enabling new research avenues, targeting microbial metabolism and 'nutritional virulence' strategies, or the respective host degradation/biosynthetic pathways exploited by the microbes, might provide novel opportunities for effective treatment of many infectious diseases by depriving microbial pathogens of essential nutrients. Interfering with host processes might cause a general risk for adverse effects but the *Anaplasma* resistance in Beclin 2-heterozygous mice (see above) reveals that partial inhibition of crucial host processes can indeed result in selective antimicrobial effects. Improved knowledge on pathogen nutritional strategies might also facilitate development of the next generation of auxotrophic mutants for efficacious vaccines for human and veterinary applications. Taken together, deciphering mechanisms of microbial 'nutritional virulence' and metabolism *in vivo* will enable comprehensive understanding of a central aspect of infectious diseases and offer novel opportunities for treatment and prevention.

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