Microreview

Intracellular innate resistance to bacterial pathogens

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Summary

Mammalian innate immunity stimulates antigen-specific immune responses and acts to control infection prior to the onset of adaptive immunity. Some bacterial pathogens replicate within the host cell and are therefore sheltered from some protective aspects of innate immunity such as complement. Here we focus on mechanisms of innate intracellular resistance encountered by bacterial pathogens and how some bacteria can evade destruction by the innate immune system. Major strategies of intracellular antibacterial defence include pathogen compartmentalization and iron limitation. Compartmentalization of pathogens within the host endocytic pathway is critical for generating high local concentrations of antimicrobial molecules, such as reactive oxygen species, and regulating concentrations of divalent cations that are essential for microbial growth. Cytosolic sensing, autophagy, sequestration of essential nutrients and membrane attack by antimicrobial peptides are also discussed.

Introduction

The mammalian innate immune system serves as a powerful barrier to invading bacterial pathogens by employing direct antimicrobial mechanisms, and indirectly, by stimulating the potent and antigen-specific adaptive immune response. As the peak of primary adaptive immunity, characterized by lymphocyte activation and proliferation, does not occur until 5–7 days post infection, the innate immune system must employ resistance mechanisms that control bacterial survival and replication. Successful pathogens, such as *Mycobacterium tuberculosis*, by definition are able to cause disease in spite of innate immune defences. However, the innate immune system is likely protective

against many opportunistic pathogens and may increase the infectious dose of a successful pathogen required for symptomatic disease to occur. The study of host–pathogen interactions has been instrumental in defining pathways of innate immune defence. This review will focus on innate antimicrobial mechanisms encountered by bacteria that enter the intracellular niche (Table 1). Most intracellular bacterial pathogens target macrophages or epithelial cells for entry, therefore we will primarily discuss innate immune strategies within these cell types. Bacteria may enter mammalian cells by phagocytosis or by facilitating their own uptake, termed invasion. During the process of phagocytosis or invasion, innate immune sensors like the Toll-like receptors (TLRs) or Nod-like receptors (NLRs) may be triggered by bacterial ligands. The signalling pathways activated by TLRs and NLRs have been extensively reviewed elsewhere and predominantly lead to expression and activation of proinflammatory cytokines such as IL-1β (Inohara *et al.*, 2005; Beutler *et al.*, 2006). These receptor networks perform a critical but largely paracrine role in innate resistance to intracellular bacteria by allowing infected cells to communicate with other effectors of innate and adaptive immunity. However, some proteins of the TLR and NLR families, as well as other mechanisms discussed later, play important and direct roles within infected cells to mediate resistance to intracellular bacteria.

Once inside the host cell, bacteria face a formidable battery of defences in the endosome or the phagosome, a specialized vesicle of the endocytic compartment. Pathogenic bacteria have evolved ways to avoid these innate defences either by diverting normal vesicular trafficking or by escaping into the host cytosol (Fig. 1). Although less well understood, a number of recent studies have demonstrated that innate antibacterial mechanisms also exist in the mammalian cytosol. However, the predominant strategy in innate immunity to intracellular bacterial pathogens appears to involve compartmentalization within membrane bound vacuoles (Fig. 2). Compartmentalization serves multiple purposes in the innate immune response. First, the physical barrier of the bacteria-containing vacuole provides an enclosed space within which the host cell can direct a potent antimicrobial attack such as the oxidative burst. Host enzymes that mediate antibacterial activity might also compromise essential

Table 1. Model intracellular bacterial pathogens.

Pathogen	Host cell – compartment	Major mechanisms of innate immune evasion
Chlamydia trachomatis	Epithelial cell – chlamydial inclusion	Avoids lysosome fusion by diverting chlamydial inclusion from normal vesicular trafficking pathway (Hackstadt et al., 1997)
Coxiella burnetii	Macrophage, epithelial cell, fibroblast – acidic vacuole with markers of autophagy	Exploits autophagic pathway, acid tolerant (Raoult et al., 1990; Gutierrez et al., 2005)
Legionella pneumophila	Macrophage – initially found in autophagosome, then in lysosome	Modulates autophagic pathway, delays fusion with lysosomes (Andrews et al., 1998; Wiater et al., 1998; Amer and Swanson, 2005;)
Listeria monocytogenes	Macrophage, epithelial cell, fibroblast – cytosol	Delays fusion with lysosomes, escapes from phagosome (Gaillard et al., 1987; Tilney and Portnoy, 1989; Henry et al., 2006)
Mycobacterium tuberculosis	Macrophage – modified early endosome	Inhibits phagolysosome fusion, resistant to RNI (Flynn and Chan, 2003)
Salmonella enterica ser. Typhimurium	Macrophage, epithelial cell – *Salmonella*-containing vacuole (acquires LAMP1 but inhibits fusion with lysosomes)	Modulates fusion with lysosomes, inhibits recruitment of phagocyte oxidase, resistance to antimicrobial peptides (Vazquez-Torres et al., 2000; Ernst et al., 2001; Holden, 2002)
Shigella flexneri	Epithelial cell, fibroblast – cytosol	Escapes from phagosome, inhibits autophagy (High et al., 1992; Ogawa et al., 2005)

host cell functions if their activity were not confined to the phagosome. Second, the bacteria are contained within an environment that has low nutrient availability compared with the host cytosol. Lastly, while not the focus of this review, containment of bacteria within the endocytic pathway facilitates MHC Class II antigen presentation by professional antigen presenting cells, such as dendritic cells, which stimulate antigen specific adaptive immunity.

Fusion with lysosomes

Bacterial invasion results in compartmentalization within an endosome. In a highly regulated manner, endosomes fuse with late endosomes and lysosomes which have an acidified lumen based on the proton pump activity of the vacuolar ATPase (vATPase) (Mellman et al., 1986; Luzio et al., 2003). Inhibition of vATPase activity by the macrolide, Bafilomycin A, decreases the antimicrobial activity of alveolar macrophages (Bidani et al., 2000). Almost all intracellular pathogens inhibit or delay lysosome fusion suggesting that this cellular process must be subverted for bacteria to successfully establish a replicative environment (Table 1). *Salmonella enterica* ser. Typhimurium (*Salmonella typhimurium*) largely avoids lysosomes; under culture conditions where fusion of the *Salmonella*-containing vacuole (SCV) to the lysosome was increased, the bacteria were killed much more efficiently highlighting the importance of the harsh lysosomal environment as a mechanism of innate immune defence (Mukherjee et al., 2002).

Many published reports show that lysosomes have the capacity to kill and degrade bacteria, however, the exact process by which bacteria are destroyed is multifactorial and somewhat ill defined. Lysosomal pH reaches ~4.8 which is damaging to non-acid tolerant bacteria, and provides an optimal environment for lytic enzymes present in the lysosome which include lipases, lysozyme and proteases. It was recently reported that mice deficient in the lysosomal aspartyl protease cathepsin D (CtsD) showed increased susceptibility to *Listeria monocytogenes* infection, and bacterial growth and survival was enhanced in $CtsD^{-/-}$ fibroblasts and macrophages (del Cerro-Vadillo et al., 2006). Many lysosomal proteins, such as cathepsin G, have demonstrated antibacterial activity *in vitro* in purified form, but their individual roles in defence against intracellular bacteria have not been rigorously tested (Rivera-Marrero et al., 2004). Interestingly, some lytic enzymes including cathepsin G, can kill bacteria *in vitro* independently of their catalytic function (Shafer et al., 1996). These proteins contain alpha helical domains that are proposed to act similarly to small cationic antimicrobial peptides (AMP; discussed below).

Lysozyme, a muramidase that degrades bacterial peptidoglycan (PG), is another lytic enzyme found in phagolysosomes that may serve multiple intracellular
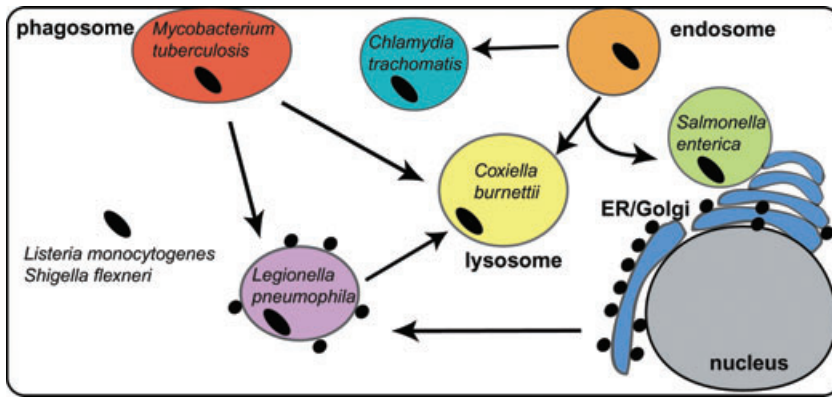
Fig. 1. Exploitation of the intracellular niche by bacterial pathogens. Bacteria can enter cells by invasion or phagocytosis. Once inside the cell, these bacteria can be found in an endosome, or in a specialized vesicle termed the phagosome. Particles internalized into endosomes traffic to the lysosomal compartment; however, many bacterial pathogens inhibit fusion with lysosomes or deviate entirely from endocytic trafficking by establishing an isolated vacuole or by escaping into the cytosol.

innate immune functions (Miyauchi *et al.*, 1985; Radons *et al.*, 1994). First, weakening of the cell wall structure, particularly in Gram-positive bacteria which have a thick PG layer, may allow better access of AMP to the bacterial membrane. *In vitro*, lysozyme significantly decreased the concentrations of AMP required for bacterial killing (Yan and Hancock, 2001; Chen *et al.*, 2005). Second, release of muramyl peptides from the bacterial cell wall by lysozyme in combination with other bacterial or host lytic enzymes may provide ligands for recognition by host pattern recognition receptors that can regulate inflammatory responses (Lenz *et al.*, 2003; Strober *et al.*, 2006). In support of this idea, mice deficient in Lysosome M, the homologue of human lysozyme, exhibited an altered inflammatory response compared with control mice in response to subcutaneous injection of

PG (Ganz *et al.*, 2003). Lastly, lysozyme is reported to have antibacterial function that is independent of its muramidase activity (Nash *et al.*, 2006). Thus, it is likely that lysozyme contributes to innate resistance during infection by intracellular bacterial pathogens, but to date there are no reports in the literature empirically testing this hypothesis. *LysM*$^{-/-}$ mice were more susceptible than their wild-type littermates to infection by extracellular bacteria; future studies using intracellular bacterial pathogens to infect *lysM*$^{-/-}$ mice will likely yield a definitive conclusion as to whether lysozyme is a key player in innate resistance to this class of pathogens (Ganz *et al.*, 2003; Cole *et al.*, 2005).

Immune regulation of vesicular trafficking occurs during bacterial infection to enhance intrinsic fusion of vacuoles containing bacteria with lysosomes, although these
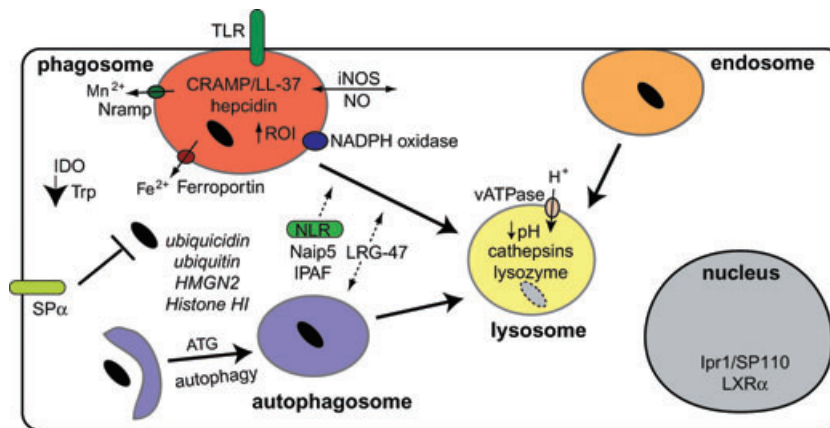

Fig. 2. Cell autonomous mechanisms of innate resistance to intracellular bacterial pathogens. Sequential fusion of endosomes with late endosomes and lysosomes results in bacterial killing by lytic enzymes and acidic pH. Activated macrophages generate a rapid burst of reactive oxygen intermediates (ROI) in the phagosome by the NADPH oxidase followed by a prolonged production of nitric oxide (NO). Multiple efflux pumps remove divalent metal cations from the lumen of the phagosome, decreasing the ability of pathogens to use metal-dependent enzymes to remediate oxidative stress or for metabolism. The phagosome also contains antimicrobial peptides, such as CRAMP. Although regulatory proteins such as LRG-47 enhance trafficking to lysosomes, some bacteria perforate the phagosome and enter the cytosol prior to phagolysosome fusion. Bacteria in the cytosol may re-enter the endocytic compartment through capture by autophagy (regulated by a signalling cascade denoted ATG) or may be subjected to putative cytosolic antimicrobial peptides (italics). Host cells also express IDO in an IFN-dependent manner to catabolize cellular pools of tryptophan which may become limiting for some bacterial species. Other proteins that contribute to innate resistance to intracellular bacteria have been identified (IPAF, Naip5, SPα, Ipr1), but exactly how these proteins mediate their antimicrobial effects is not yet known.

mechanisms are incompletely understood. An IFN-γ induced GTPase, termed LRG-47, contributes to innate resistance to *M. tuberculosis* (MacMicking *et al.*, 2003). In *lrg47$^{-/-}$* macrophages, mycobacterial phagosomes showed impaired fusion with lysosomes, decreased levels of vATPase and exhibited a higher pH than wild-type macrophages, indicating that LRG-47 directly or indirectly regulates vesicular trafficking. Thus, the ability of the host cell to restrict intracellular bacteria to the endocytic compartment and regulate the nature of the vacuolar environment subjects invading bacteria to attack by damaging conditions such as low pH, and restricts nutrient access while protecting normal host function.

Reactive oxygen and nitrogen intermediates

Professional phagocytes such as macrophages and neutrophils take up bacteria into a specialized vesicle known as the phagosome which is the target for many innate defence mechanisms. A particularly powerful host defence is the oxidative burst produced by the NADPH-dependent phagocyte oxidase. Humans with a non-functional phagocyte oxidase exhibit immunodeficiency characterized by recurrent bacterial and fungal infections (Baehner and Nathan, 1967). In resting cells, the phagocyte oxidase complex is present but dissociated and inactive; one component, gp91^{phox} (flavocytochrome b$_{558}$), is located in the plasma membrane. Upon phagocytosis, the cytosolic components, p47^{phox}, p67^{phox} and p40^{phox}, translocate to the phagosome in a Rac-dependent manner and facilitate the formation of superoxide within the lumen of the phagosome (Nauseef, 2004). *S. typhimurium* interferes with recruitment of oxidase components to the phagosome via the SPI-2 Type III secretion system (T3SS) (Vazquez-Torres *et al.*, 2000). In the absence of a functional SPI-2 T3SS, wild-type but not *phox*-deficient macrophages are able to restrict *S. typhimurium* infection demonstrating a critical role for the NAPDH oxidase in controlling intracellular bacterial growth.

Bacterial infection also stimulates the production of reactive nitrogen intermediates (RNI) by the inducible nitric oxide synthase (iNOS). *Nos2*, the gene encoding iNOS, is not transcribed in uninfected cells, but can be induced by Type I and Type II interferons; therefore, nitric oxide (NO) production is not an immediate response to infection. However, NO, unlike reactive oxygen species, diffuses across membranes and can act upon bacteria in both vacuoles and the cytosol over a longer period of time than the short-lived oxidative burst. Mice deficient in iNOS are more susceptible to infection by many intracellular pathogens than their wild-type counterparts (Chakravortty and Hensel, 2003). The role of iNOS in human macrophages has been more controversial. Some groups reported little or no detectable expression of iNOS in cultured

human macrophages, but other studies showed that macrophages isolated from tuberculosis patients did express iNOS, and iNOS function was critical in these cells during *in vitro* infection (Nathan, 2006). In mice, RNI and reactive oxygen intermediates (ROI) can synergize in eliminating the intracellular pathogens *S. typhimurium* and *L. monocytogenes* as *gp91$^{phox-/-}$nos2$^{-/-}$* mice are highly susceptible to infections by these bacteria (Shiloh *et al.*, 1999). However, cell culture infection of macrophages deficient in the phagocyte oxidase, iNOS or both, indicated that production of ROI was more critical in short-term bacterial killing. ROI and RNI also prevent escape of viable *L. monocytogenes* from the phagosome emphasizing that these molecules also contribute to innate resistance by mechanisms other than direct bacterial killing (Myers *et al.*, 2003).

Antimicrobial peptides

Small cationic peptides, or AMP produced by diverse host organisms can act as powerful antibacterial agents (Yang *et al.*, 2004). These helical peptides are thought to preferentially disrupt bacterial membranes leading to loss of membrane potential and bacterial viability. AMP are primarily secreted proteins; the major sites of AMP activity are mucosal surfaces which can be portals for bacterial entry. Neutrophils also contain many granule associated AMP and proteins that are released by exocytosis to kill extracellular bacteria or released into phagosomes to destroy phagocytosed bacteria; these two types of killing are not often experimentally distinct (Faurschou and Borregaard, 2003). There are few AMP that are definitively known to act upon bacteria primarily within macrophages and epithelial cells. Here we discuss examples of AMP that may be encountered by intracellular bacterial pathogens. One vacuolar AMP from mouse myeloid cells has been identified, cathelicidin-related antimicrobial peptide (CRAMP; homologue of human LL-37), that contributes to innate resistance to *S. typhimurium* infection (Gallo *et al.*, 1997; Rosenberger and Finlay, 2002; Rosenberger *et al.*, 2004). Induction of CRAMP expression requires generation of reactive oxygen, implicating ROI in innate immune signalling cascades as well as in direct antimicrobial functions. In human monocytes, triggering of the TLR2/1 heterodimer activated a vitamin D3-dependent induction of the cationic peptide LL-37 (the active processed form of cathelicidin, human homologue of CRAMP) (Liu *et al.*, 2006). Colocalization of cathelicidin with vacuoles containing M. *tuberculosis* was also observed concomitant with a decrease in bacterial viability. In addition to cathelicidin, other lysosomal proteins like Cathepsin G and lysozyme contain positively charged helical domains that are proposed to disrupt bacterial membranes similarly to small cationic peptides.

Taken together, these studies show that AMP play a role in innate resistance to intracellular pathogens by acting on bacteria sequestered within vacuolar compartments.

Vacuolar AMP would be effective against intracellular bacteria that replicate in a membrane bound compartment but would likely have limited efficacy against cytosolic pathogens such as *L. monocytogenes* which quickly escapes from the phagosome. Several groups have identified cytosolic components, namely ubiquitin, ubiquicidin, histones and HMGN2, that have antimicrobial activity *in vitro*, but as yet, it is unclear what role these proteins play during intracellular infection. Ubiquitin is a small polypeptide that can be covalently linked to proteins to regulate signalling, endocytosis or degradation. It was recently shown that ubiquitin derived peptides exhibited antibacterial activity *in vitro* (Kieffer *et al.*, 2003). A distinct small polypeptide, ubiquicidin, was originally characterized biochemically as an antimicrobial activity isolated from the cytosol of IFN-γ treated mouse macrophages (Hiemstra *et al.*, 1999). Further analysis showed identity with the small ribosomal subunit, S30. Ubiquicidin has potent antimicrobial activity against *L. monocytogenes* and *S. Typhimurium in vitro*. However no depletion or inhibition experiments have been performed to show that the peptide functions to kill intracellular bacteria in infected macrophages or mice; the interpretation of such experiments might be complicated by non-specific effects of ubiquicidin/S30 depletion on normal cellular processes. This problem is reminiscent of the dilemma associated with the discovery of the antimicrobial function of histones many decades ago (Hirsch, 1958). While the *in vitro* antibacterial activity of histones was unquestioned, it was thought to be non-specific and due to the highly basic nature of histones that facilitate binding to DNA. Thus, it was unclear whether histones had any physiological role in the immune response to bacterial infections. Recently, histones and DNA were shown to participate in bacterial killing by neutrophil extracellular traps, or NETs, where it is unlikely that histones are required solely for participation in chromatin structure and transcriptional regulation (Brinkmann *et al.*, 2004; Beiter *et al.*, 2006; Buchanan *et al.*, 2006). Histones are found in the mammalian cytosol as well as the nucleus and therefore may also contribute to controlling cytosolic bacterial pathogens (Zlatanova *et al.*, 1990). Similarly, another DNA binding protein HMGN2, also reported to have antimicrobial activity, is present in the cytosol and can be secreted from human mononuclear leukocytes (Feng *et al.*, 2005). Host cells may profit both metabolically and immunologically by exploiting the biochemical properties of abundant and readily available proteins such as histones to kill bacteria that escape from the endocytic compartment.

Autophagy

Escape of bacteria into the host cytosol, the storehouse for many host nutrients, presents a significant threat to the host. Most characterized mechanisms for innate resistance to bacterial pathogens are directed within membrane bound compartments, but host cells do have a mechanism, termed autophagy, to capture particles in the cytosol and surround them with a newly formed membrane. The process and regulation of autophagy is conserved from yeast to man and has been primarily studied in the context of the cellular response to stress and starvation (Shintani and Klionsky, 2004). However, recent studies clearly show an important role for autophagy in capturing and eliminating bacteria in the cytosol (Rich *et al.*, 2003; Nakagawa *et al.*, 2004). The cytosolic pathogen, *Shigella flexneri,* actively inhibits autophagy; a strain deficient in the virulence factor IcsB was found trapped in autophagosomes (Ogawa *et al.*, 2005). Moreover, autophagy facilitates elimination of pathogen-containing vacuoles by fusion with lysosomes. This process is enhanced by the IFN-γ inducible GTPase LRG-47 in the case of *M. tuberculosis* infection (Gutierrez *et al.*, 2004; Birmingham *et al.*, 2006). It is as yet unclear how autophagy is initiated by bacterial infection although phosphoinositides participate in the regulatory process; vacuolar membrane damage has been proposed as a possible trigger (Birmingham *et al.*, 2006; Lindmo and Stenmark, 2006). In the case of *Legionella pneumophila*, macrophage regulation of autophagy may mediate the difference between resistance and permissiveness. Resistant C57Bl/6 macrophages exhibit more rapid maturation of pathogen containing autophagosomes than susceptible A/J macrophages which lack normal levels of Naip5 (Amer and Swanson, 2005). Autophagy is upregulated by IFN-γ treatment like many other mechanisms of innate resistance. In addition to a direct antimicrobial function, autophagy also allows infected cells to capture antigens from cytosolic pathogens for processing and presentation on MHC Class II (Deretic, 2005; Munz, 2006).

Nutritional Immunity

The host cell by necessity must contain all of the nutrients required for its own metabolism, however, these stores also are a rich source for bacterial pathogens to exploit. Thus, another major strategy for innate resistance to intracellular infection is to sequester key nutrients such as iron from the invading bacteria. Iron is essential for many vital processes of the host and bacterium, including metabolism and protection from oxidative stress. Although iron is present in host cells, it is predominantly bound to proteins and is virtually unavailable in free soluble form. Upon phagocytosis of a bacterium, iron can be removed from

the phagosome via multiple mechanisms. First, ferroportin, Nramp1 and Nramp2 (DMT1) act as iron transporters to move ferrous iron from the lumen of the phagosome into the cytosol (Forbes and Gros, 2003; Chlosta *et al.*, 2006). Nramp1 also acts as a manganese efflux pump; both manganese and iron can be used as co-ordinating metal centres for bacterial proteins involved in protection against oxidative stress such as superoxide dismutase (Jabado *et al.*, 2000; Papp-Wallace and Maguire, 2006). Second, expression of transferrin receptor, a high affinity receptor for iron that allows iron uptake from plasma, is downregulated in response to IFN-γ which decreases the amount of iron in the phagosome (Byrd and Horwitz, 1989; 1993). *Coxiella burnetii* overcomes the iron deficit by stimulating upregulation of transferrin receptor (Howe and Mallavia, 1999). Third, lactoferrin, an iron chelator found in neutrophil granules, can be taken up into the macrophage endocytic pathway from the extracellular milieu and thereby decrease the concentration of available iron in the phagosome (Byrd and Horwitz, 1991). Hepcidin, a small AMP which is essential for iron homeostasis during normal host metabolism, also acts to increase iron efflux in macrophages (Peyssonnaux *et al.*, 2006). Both Fe^{2+} and Mn^{2+} are critical for virulence of many pathogens, such as *S. typhimurium* (Boyer *et al.*, 2002). To enable replication, many intracellular bacteria produce high affinity iron chelators, called siderophores, which are able to steal iron from host proteins (De Voss *et al.*, 2000; Parent *et al.*, 2002; Fischbach *et al.*, 2006). Host cells have in turn evolved innate immune mechanisms to protect iron acquisition by siderophores. Lipocalin-2, which is upregulated by TLR signalling, binds siderophores of the enterobactin family produced by Gram negative bacteria like *S. typhimurium* (Flo *et al.*, 2004). Thus, regulation of iron availability is central in determining the balance of the host–pathogen interaction.

Intracellular bacteria must also acquire many other nutrients to promote survival and replication. Global gene expression analysis has revealed that compared with extracellular counterparts, intracellular bacteria upregulate genes characteristic of nutritional stress such as iron, magnesium, glucose, amino acid and phosphate limitation (Chatterjee *et al.*, 2006; Jansen and Yu, 2006). By analogy to the extensive regulation of iron availability, it is reasonable to speculate that other nutrients may be sequestered by the host cell during infection, but few relevant mechanisms have been described. One mechanism of nutrient deprivation has been well established – tryptophan catabolism by indoleamine 2,3-dioxygenase (IDO). IDO has two functions in innate immunity – a direct role in nutrient deprivation during intracellular infection and a role in negative regulation of T cell activation (Thomas *et al.*, 1993; Beatty *et al.*, 1994; Mellor and Munn, 2004). Upregulation of IDO by IFN-γ restricted

growth of *Chlamydia trachomatis*; the bacteriostatic effect was abrogated by inhibition or deficiency of IDO or by addition of excess tryptophan. Interfering with bacterial metabolism is a powerful strategy to restrict bacterial growth. Future studies may identify additional mechanisms by which host cells modulate nutrient availability.

Future questions

Innate immune control of intracellular bacterial pathogens depends on diverse, overlapping and redundant mechanisms to ensure survival of the host until the onset of adaptive immunity. While innate immune recognition and signalling by TLR and NLR proteins have been explored in exquisite molecular detail, how these sensors may trigger direct antimicrobial mechanisms is for the most part poorly defined. Two members of the NLR family, Naip5 (also called Birc1e) and IPAF (also termed CLAN), which recognize cytosolic bacterial flagellin, exert a protective effect against macrophage infection by *L. pneumophila* and *S. typhimurium* (Diez *et al.*, 2003; Wright *et al.*, 2003; Molofsky *et al.*, 2006; Ren *et al.*, 2006; Zamboni *et al.*, 2006). The bacterial flagellin likely contaminates the host cytosol via bacterial secretion systems, such as the Type III or Type IV systems, that translocate virulence factors across the vacuolar membrane. These data demonstrate that Naip5 and IPAF contribute to important mechanisms of bacterial control but exactly how is unclear. It is paradoxical that cytosolic detection systems act to inhibit growth of vacuolar pathogens but they may serve to prevent inappropriate immune responses to nonpathogenic vacuole-bound bacteria that present no danger to the host. As there are members of the NLR family that have not yet been extensively investigated, the possibility remains that some additional NLRs will also be important in preventing bacterial replication, while others, such as Nod1, may function primarily as regulators of inflammation.

Other regulators of innate immune control of bacterial replication that remain to be explored are Ipr1, LXRα and SPα. Ipr1, an interferon inducible gene, was identified genetically as a locus that controls resistance to *M. tuberculosis* infection in macrophages, mice and possibly humans (Pan *et al.*, 2005; Tosh *et al.*, 2006). The closest human homologue to Ipr1 is SP110b, a protein that contains chromatin binding and nuclear localization domains, which may act as a cofactor for nuclear receptors to regulate transcription (Bloch *et al.*, 2000). Tontonoz and colleagues reported that LXRα, a nuclear receptor of the liver X receptor family, regulates cholesterol metabolism and resistance to the cytosolic pathogen, *L. monocytogenes* (Joseph *et al.*, 2004). SPα, a scavenger receptor cystine rich repeat protein, was identified as an LXRα target gene that contributed to antibacterial activity. SPα

has been implicated in protection from apoptosis, but a recent study suggested that SPα could also act as a pattern recognition receptor for lipopolysaccharide (LPS) (Sarrias *et al.*, 2005). Because *L. monocytogenes* has no LPS, the defect in antibacterial function associated with the absence of LXRα and SPα in *L. monocytogenes* infection cannot be explained by LPS recognition but implicates some other role of SPα yet to be determined. It is apparent that we have much to learn about the many mechanisms by which host cells orchestrate innate immune control of bacterial pathogens that exploit the intracellular niche. An increased understanding of intracellular innate immune resistance may provide important clues about how to design more effective vaccines and therapeutic strategies to combat bacterial pathogens.

Acknowledgements

Note added in proof

Miao *et al.* have also identified IPAF as a sensor for cytosolic flagellin. Miao EA, Alpuche-Aranda CM, Dors M, Clark AE, Bader MW, Miller SI, Aderem A. (2006) Cytoplasmic flagellin activates caspase-1 and secretion of interleukin I beta via Ipaf. *Nat Immunol* **7**:569–75.

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