Invasion of eukaryotic cells by *Legionella pneumophila*: A common strategy for all hosts?

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PS HOFFMAN. Invasion of eukaryotic cells by *Legionella pneumophila*: A common strategy for all hosts? Can J Infect Dis 1997:8(3):139-146. *Legionella pneumophila* is an environmental micro-organism capable of producing an acute lobar pneumonia, commonly referred to as Legionnaires' disease, in susceptible humans. Legionellae are ubiquitous in aquatic environments, where they survive in biofilms or intracellularly in various protozoans. Susceptible humans become infected by breathing aerosols laden with the bacteria. The target cell for human infection is the alveolar macrophage, in which the bacteria abrogate phagolysosomal fusion. The remarkable ability of *L pneumophila* to infect a wide range of eukaryotic cells suggests a common strategy that exploits very fundamental cellular processes. The bacteria enter host cells via coiling phagocytosis and quickly subvert organelle trafficking events, leading to formation of a replicative phagosome in which the bacteria multiply. Vegetative growth continues for 8 to 10 h, after which the bacteria develop into a short, highly motile form called the 'mature form'. The mature form exhibits a thickening of the cell wall, stains red with the Gimenez stain, and is between 10 and 100 times more infectious than agar-grown bacteria. Following host cell lysis, the released bacteria infect other host cells, in which the mature form differentiates into a Gimenez-negative vegetative form, and the cycle begins anew. Virulence of *L pneumophila* is considered to be multifactorial, and there is growing evidence for both stage specific and sequential gene expression. Thus, *L pneumophila* may be a good model system for dissecting events associated with the host-parasite interactions.

Key words*: Intracellular parasites,* Legionella pneumophila*, Organelle trafficking, Pathogenesis, Stress proteins*

Invasion de cellules eucaryotes par *Legionella pneumophila* : stratégie commune pour tous les hôtes?

RÉSUMÉ : *Legionella pneumophila* est un microorganisme environnemental capable de produire une pneumonie lobulaire aiguë appelée maladie du Légionnaire chez des sujets sensibles. Le genre *Legionella* est abondant dans les milieux aquatiques où il survit en biofilms ou intracellulairement dans divers protozoaires. Les sujets humains qui y sont sensibles deviennent infectés en respirant la bactérie présente dans l'air. La cellule cible chez l'être humain est le macrophage alvéolaire dans lequel les bactéries abrogent la fusion phagolysosomique. La capacité remarquable de *L. pneumophila* à infecter une grande variété de cellules eucaryotes suggère l'existence d'une stratégie commune qui exploite tous les processus cellulaires fondamentaux. La bactérie pénètre les cellules de l'hôte par la phagocytose en hélice et perturbe rapidement le fonctionnement des organelles, entraînant la formation d'un phagosome réplicant dans lequel la bactérie se multiplie. La croissance végétative se poursuit pendant huit à dix heures, après quoi la bactérie adopte la forme courte et hautement motile de l'organisme à maturité. Ce dernier manifeste un épaississement de la paroi cellulaire, prend une teinte rouge à la coloration de Gimenez et serait de 10 à 100 fois plus infectieux que la bactérie mise en croissance sur gélose. Après la lyse des cellules de l'hôte, les bactéries sécrétées infectent d'autres cellules de l'hôte dans lesquelles les cellules à maturité se différencient en une forme végétative Gimenez-négative et le cycle reprend. La virulence de *L. pneumophila* serait plurifactorielle et les preuves s'accumulent au sujet de l'existence d'une expression génique spécifique au stade et séquentielle. Ainsi, *L. pneumophila* peut être un bon modèle pour l'étude des événements associés aux interactions hôtes-parasites.

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The summer of 1996 marked the 20th anniversary of the original outbreak of Legionnaires' disease that followed an American Legion convention in Philadelphia, Pennsylvania in 1976. Neither identification of the agent nor the etiology of disease were forthcoming, leading to much concern which was spurred on by media hype and sensationalism. Few were able to predict, however, that Legionnaires' disease would only be a harbinger of diseases to come, such as toxic shock, AIDS and multiple-drug resistance. By the end of 1976, Joe McDade at the Centers for Disease Control, Atlanta, Georgia, had identified the culprit, a Gram-negative aerobic rod-shaped bacterium subsequently named *Legionella pneumophila*, in memory of the Legionnaires' who died in the original epidemic (1). The species name reflects the human disease, an acute lobar pneumonia.

The past twenty years has seen the development of good diagnostic and therapeutic procedures, and the identification and management of potential sources of infection, including cooling towers and potable water systems. Through the tools of molecular genetics and cell biology, we have begun to dissect virulence strategies and the specifics of the host cellular immune response against this intracellular parasite. Yet, despite all this knowledge and technology, Legionnaires' disease is still problematic, accounting for nearly 4% of all community and nosocomial pneumonia (2). The incidence of Legionnaires' disease in the United States is estimated to be 20,000 cases per year (3). Those at greatest risk of acquiring legionellosis include immunocompromised individuals (4), including cardiac transplant patients (5). Explosive outbreaks can still be traced to improperly maintained cooling towers, hot tubs and dehumidifiers (1,6).

L pneumophila is primarily responsible for most cases of legionellosis, with other species accounting for about 10% of cases (7). *Legionella* species are found in aquatic habitats and moist soils, where they may live freely in biofilms or intracellularly in a variety of protozoa, including *Hartmanella, Acanthamoeba* and *Naeglaria* species(8-11). Protozoa may serve as a biological amplifier for these bacteria in cooling towers and hot water systems (8). Humans become infected by breathing aerosols laden with legionella bacteria. Interestingly, protozoans in aerosols may even potentiate infection (12). Early studies quickly identified the alveolar macrophage as the target cell colonized by *L pneumophila* (13). Moreover, it was noted that in vitro *L pneumophila* altered events in macrophages that ordinarily would lead to fusion of secondary lysosomes with bacteria-laden phagosomes (14).

Legionella bacteria remain in the phagosome, which does not fuse with secondary lysosomes or become acidified (15). In this replicative phagosome, the bacteria quickly multiply and eventually lyse the host cell (16). Thus, the macrophage became the model system used for dissecting events associated with invasion and intracellular growth (17-21). More recently, biologists have considered what *L pneumophila* gains from human infection, and, surprisingly, the answer is not very much. In fact, humans represent a mistake in the survival strategy of the bacteria because, unlike tuberculosis, the common cold or whooping cough, legionellosis is not a communicable disease. Legionnaires' disease should be viewed as an inadvertent outcome of the bacteria's attempt to colonize macrophages instead of its natural aquatic hosts. This review will focus on recent developments that support a common strategy used by *L pneumophila* for the invasion and subversion of host cells, a prerequisite for bacterial multiplication. Readers are referred to other reviews for additional information on the history of legionnaires disease (22), virulence factors (23), genetics (24,25) and immunology (26).

INVASION OF HOST CELLS

Because both professional and nonprofessional phagocytes can serve as hosts for *L pneumophila*, it is reasonable to expect that invasion may be mediated through several mechanisms. In the case of professional phagocytes (alveolar macrophages, monocytes and neutrophils), complement and antibody opsonized bacteria are internalized following binding to host cell complement (CR1 and CR3) or Fc integrin receptors (27-29). These activities signal cytoskeletal rearrangements that result in internalization of the bacteria. In fact, *L pneumophila* is so rapidly internalized by professional phagocytes that there has been little interest until recently to examine invasion mechanisms further. However, the significance of complement and antibody in phagocytosis has recently been challenged by Gibson et al (30), who found that the addition of these components to various host cell invasion assays had little or no effect on the invasion kinetics. Because nonprofessional phagocytes (epithelial cells and fibroblast cells) are also invaded by *L pneumophila*, it has been suggested that invasion must be parasite-directed. This latter mechanism may have significance for human infection because several studies indicate that lung epithelial cells might be involved in legionella pathogenesis (31,32). The current author's studies with HeLa cells support a parasite-directed mechanism because HeLa cells are not naturally phagocytic and lack complement receptors (33,34). Virulent strains of *L pneumophila* are 100 to 1000 times more invasive than avirulent strains, suggesting that avirulent strains may be deficient in expression of surface molecules required for initiating parasite-directed endocytosis. The observed differences are not due to attachment. The greater awareness that natural hosts do not rely on complement or antibodies to aid the phagocytic process has rekindled interest in identifying surface proteins that likely play a role in parasite-directed endocytosis.

Parasite-directed endocytosis is a common mechanism in the microbial world, one used by other invasive bacteria including *Listeria monocytogenes* (35), *Yersinia* species (36,37), *Salmonella* species (38), *Shigella* species and enteroinvasive strains of *Escherichia coli* (38,39). In these organisms, specific surface or outer membrane proteins are involved in invasion. In contrast to most Gram-negative bacteria, *L pneumophila* expresses relatively few outer membrane proteins (40). The major outer membrane protein, OmpS, is a trimeric porin composed of two 28.5 kDa subunits and one 31 kDa subunit, the latter being bound covalently via an N-terminal glycine to the peptidoglycan (41,42). The covalent bonding of OmpS to the peptidoglycan is unique to *L pneumophila* and *Legionella*

micdadei, but might have parallels to the major outer membrane protein (MOMP) of *Chlamydia* species for which the porin is bound to other proteins via inter-chain disulfide bonds (43). Similarly, the porin of *L pneumophila* is cross-linked via inter-chain disulfide bonds (42). Antibody raised to OmpS subunits blocks attachment of *L pneumophila* to HeLa cells, suggesting a role for OmpS in pathogenesis (44). Because the surface integrins of eucaryotic cells often contain thiol groups essential for biological activity (45), it is not unreasonable to predict that future studies might show OmpS thiol groups interact with the thiol groups of surface integrin proteins. Integrin proteins are common to all eucaryotic cells and, therefore, may serve as a common target for bacterial adhesion. Other proteins in the outer membrane include a 19 kDa lipoprotein (46) and a 24 kDa prolyl-isomerase macrophage invasion potentiator protein (MIP) (47). Neither of these proteins are essential for virulence, although knockout mutations in MIP affect invasion (48,49). All proteins mentioned herein are also expressed by avirulent mutants, leading to the conclusion that these proteins, while potentially functioning in adhesion, are probably not the invasin responsible for internalization by HeLa cells.

ROLE OF SURFACE AND SECRETED PROTEINS IN INVASION

For some bacteria, contact with host cells is a prerequisite for induction of genes encoding invasin proteins (50). In the case of *L pneumophila*, seminal studies conducted in Horwitz's laboratory (51) demonstrated that both invasion and abrogation of phagosome lysosome fusion occur in the presence of antibiotics such as erythromycin, indicating that the proteins responsible for these events must preexist on the surface of agar-grown virulent strains. In these studies, internalized bacteria were not killed by the infected monocytes, and bacterial growth resumed upon removal of the antibiotic. The ability of *L pneumophila* to survive in phagocytic cells in the presence of antibiotics has clinical significance because eradication of infection in immunocompromised patients is both difficult and requires extended treatment regimens (2,5).

One protein that appears to be differentially expressed on the bacterial surface between virulent and avirulent strains is Hsp60, a member of the GroEL family of heat shock proteins (33,52). Studies have shown that the GroEL family of proteins is highly conserved through evolution and is essential for bacterial viability. Fernandez et al (53) demonstrated that Hsp60 was preferentially synthesized during the first hour post infection of mouse L929 cells and human monocytes. Moreover, Hsp60 accumulated in the phagosomes of host cells during this period. Unpublished work has also demonstrated that anti-Hsp60 serum blocks invasion of HeLa cells by *L pneumophila*. The addition of purified Hsp60 protein to the monolayer before the addition of bacteria also prevented invasion, suggesting that HeLa cell surface receptors were saturated by the added Hsp60 protein. These data support the hypothesis that the heat shock or stress protein Hsp60 participates in invasion. This conclusion is further supported by the observation that avirulent strains, which poorly transport Hsp60 to the bacterial surface, exhibit diminished ability to invade HeLa cells.

The Hsp60 hypothesis has merit because it fulfills two basic requirements: that the invasin be pre-existing in both virulent and avirulent strains and that the invasin be active in the virulent, but not in the avirulent strain. Because de novo protein synthesis is not required for invasion (51), the process can occur in the presence of antibiotics that block protein synthesis.

ABROGATION OF PHAGOSOME-LYSOSOME FUSION

Abrogation of phagosome lysosome fusion is a hallmark of invasion of professional phagocytes by *Legionella* species and a requirement for intracellular survival. In contrast, avirulent strains are phagocytized by monocytes but are unable to prevent phagolysosome fusion or the acidification of the vacuole (18). In general, fusion of secondary lysosomes with bacterium-laden phagosomes occurs early in the phagocytic process (54). Therefore, it is highly likely that the signal transduction cascade initiated by virulent bacteria seals the fate of the developing phagosome. One can envision that these parasite-directed interactions alter signaling functions of host cell membrane-associated kinases such that trafficking via the endocytic pathway is altered. Studies now show that the bacterium-laden phagosome is directed to the endoplasmic reticulum (55). During this process, phagosomes laden with virulent *L pneumophila* recruit vesicles, ribosomes and mitochondria. Finally, the bacterium-laden phagosome becomes surrounded by membrane material originating from the endoplasmic reticulum (55,56). Immunostaining and confocal microscopic examination show that the eucaryotic chaperone protein Bip (Hsp78) is located between the endoplasmic reticulum membrane and the phagosome. Studies by Fernandez et al (53) suggest that *L pneumophila* may contribute to the maintenance of the replicative phagosome by secreting Hsp60 into the phagosome. Immunogold electron microscopy revealed association of Hsp60 with the phagosome membranes (53). Some of this protein appears to escape the phagosome and may become associated with the endoplasmic reticulum. These studies are in their infancy, and future studies will likely begin to dissect the interactions of host and bacterial proteins in the establishment and maintenance of the replicative phagosome. Swanson and Isberg (55) provided evidence that the function of the endoplasmic reticulum was essential for intracellular replication of the bacteria because treatment of the culture with brefeldin A, which dissociates the Golgi vesicles and alters vesicle trafficking, leads to cessation of bacterial growth. Additional alterations to the replicative phagosome include hypoexpression of major histocompatibility complex molecules, which would likely affect antigen presentation (57). The exclusion of these molecules begins with attachment and internalization of virulent bacteria (58). Little is known of how legionella proteins accomplish these modifications, but the continued study of mutants that fail to grow intracellularly will likely provide some answers to these fundamental questions.

GENE EXPRESSION IN THE REPLICATIVE PHAGOSOME

Once the replicative phagosome becomes associated with the endoplasmic reticulum, it is several hours before the bacteria begin dividing. During this transition period presumably the bacteria are preparing for replication, a process that depends on the activities of many genes. Several genes appear to be expressed between 4 and 8 h postinfection and include the early stage macrophage-induced (*eml*) locus identified by differential display-polymerase chain reaction techniques (59) and a locus encoding a 44 kDa protein (60). Studies are in progress to determine whether the 44 kDa protein is a product of the *eml* locus. Selective radiolabelling of intracellular bacteria at 12 h postinfection of U937 cells has demonstrated the selective synthesis of as many as 35 proteins along with repression of another 32 proteins (61). Very little is known about intracellular nutrient acquisition by *L pneumophila*. Studies have shown that gamma interferon treated macrophages downregulate transferrin receptors, resulting in cessation of bacterial growth due to iron deprivation (62). Pope et al (63) have isolated mutants generated by random insertion of a mini *Tn*10 transposon that are defective in iron acquisition. These mutants grow poorly or not at all when in the cell. Study of these mutants will allow a better understanding of mechanisms associated with iron acquisition in the host phagosome and address the extent to which iron deprivation affects intracellular events. Nutritional studies by several groups established that *L pneumophila* required no vitamins, purines or pyrimidines, but most strains exhibited requirements for seven to nine amino acids (64). Thymine auxotrophs of *L pneumophila* are killed in the cell, indicating that in the absence of supplemented thymine this nutrient is excluded from the replicative phagosome (65). This feature has been effectively exploited by Berger and colleagues (66, 67) to identify mutants unable to grow intracellularly. In this assay, normal virulent thymine auxotrophs invade host cells, grow and then die a thymineless death. Mutants that cannot grow intracellularly survive. Genes required for intracellular growth have been identified by this very powerful selection technique (66). Two groups simultaneously identified genes – the defect in organelle trafficking (*dot*) gene (66) and the intracellular multiplication (*icm*) gene (68) – in a locus that is required for intracellular replication. These genes are adjacent and transcribed from divergent promoters (69). Some mutations in *dotA* lead to decreased virulence, whereas others lead to avirulence and tolerance to sodium chloride (67). DotA is a high molecular weight protein that spans the cytoplasmic membrane and may be involved in protein secretion. Additional *dot* genes have recently been identified that similarly span the cytoplasmic membrane, and it is speculated that these proteins may be necessary for secretion of proteins required for virulence. The *icm* locus has an operon structure, and the insertion of transposons in this region leads to the loss of virulence and tolerance to sodium chloride (69). The *icm* genes encode novel proteins, some of which may be secreted. It remains to be determined what proteins are secreted by these loci and what possible role secreted proteins play in pathogenesis.

TOLERANCE TO SODIUM CHLORIDE AND AVIRULENCE

One of the early observations by Feeley et al (70) was that the legionellae were particularly sensitive to sodium ions, leading to the recommendation that potassium hydroxide and not sodium hydroxide be used to adjust the pH of the buffered charcoal yeast extract medium used for primary isolation of the bacteria (70). This observation was studied in detail by Catrenich and Johnson (71) who found that spontaneous avirulent mutants could be selected out of a virulent population by plating the virulent bacteria on medium to which 0.6% or more sodium chloride had been added. Genetic analysis of the basis for sodium tolerance has identified as many as 16 loci, including *dot* and *icm*, that produce this phenotype (21). One gene, *ompS*, is negatively regulated by inhibitory levels of sodium chloride (53,72). Studies by Fernandez et al (53) demonstrated that synthesis of OmpS is repressed within the first hour postinfection of human macrophages and mouse L929 cells (53). Using *ompS* promoter-lacZ reporter gene fusions, Weeratna and Hoffman (unpublished data) demonstrated that in vitro challenge of virulent strains of *L pneumophila* with sodium, but not potassium, chloride led to repression of transcription as indicated by lower levels of beta-galactosidase activity post challenge. Similarly, when *L pneumophila* containing this *lacZ* construct was allowed to infect HeLa cells and subsequently assayed for beta-galactosidase activity, the activity was marginally higher than the promoterless negative control's activity. *ompS* is not expressed from an endogenous promoter in *E coli,* indicating that this highly expressed gene is under a unique transcriptional control in *L pneumophila* (73). A 15 kDa transcription factor, OmpT, has been identified as a positive regulator of *ompS* transcription. Studies with chloramphenicol established that OmpT is unstable and has a half life of less than 10 min. Similarly, when virulent strains are challenged with sodium chloride, there is a cessation of OmpT synthesis followed by rapid inactivation, as indicated by gel mobility shift experiments. In contrast with virulent strains, sodium-tolerant avirulent strains are unaffected by sodium chloride challenge. Future studies will likely lead to identification of the gene encoding OmpT, resolution as to why OmpS synthesis is repressed early in infection of host cells and whether OmpT regulates expression of additional genes.

EVIDENCE FOR A DEVELOPMENTAL CYCLE IN LEGIONELLA PATHOGENESIS

Once the replicative phagosome has been established, the bacteria begin multiplying with a doubling time of 2 h (16,17). Throughout intracellular infection, Hsp60 remains the dominant protein (58), suggesting that the protein plays an essential role in maintaining the replicative phagosome. As the replicative phagosomes become laden with bacteria (approximately 8 to 12 h postinfection), several notable morphological changes are observed. Bacteria are highly motile within the phagosome and begin to accumulate granules of the carbohydrate storage material, poly-beta-hydroxybutyrate, that accrues in high carbohydrate and low nitrogen environments (74). During this period, bacteria become shorter and begin to

accumulate intracytoplasmic membranes and vesicles (unpublished observations). As noted very early in studies of *L pneumophila* infection, the bacteria retain the Gimenez stain which agar-grown bacteria do not (23,75). This stain is commonly used for staining *Rickettsia* species and consists of carbol fuschin, a dye employed in acid-fast staining of mycobacteria. Electron microscopic examination of thin sections reveals a thickening of the outer membrane wall material with laminations of intracytoplasmic membranes. These forms are 10 times more infectious compared with agar-grown bacteria (75). Studies in the author's laboratory also show that this form is studded with Hsp60 on the bacterial cell surface, suggesting an enhanced role in adhesion or invasion.

Garduno et al (unpublished data) suggest that this form be called a 'mature form'. When the mature form is used for subsequent infection, it is indeed 10- to 100-fold more infectious than agar-grown bacteria. When the intracellular fate of these bacteria is monitored, it is noted that the bacteria loose the ability to retain the Gimenez stain during the first hour postinfection, suggesting that the mature form differentiates back into a vegetative form to begin active replication (unpublished data). The observations support the notion that *L pneumophila* indeed may have a developmental cycle. If this is true, the legionellae, like *Chlamydia* species (76), might be considered to possess both an infectious form (ie, the mature form) and a vegetative replicative form. The current author's studies also show that avirulent strains, which poorly invade HeLa cells, are able to grow in culture medium in association with the HeLa cells. The growing avirulent bacteria also weakly stain with Gimenez suggesting that, while unable to invade the HeLa cells, they are able to turn on genes that lead to development of the mature form. Many complex questions concerning regulation of developmental genes as well as responses to host and environmental signals remain to be resolved.

ARE THE LEGIONELLAE ENDOSYMBIONTS IN NATURAL HOSTS?

L pneumophila lives in aquatic habitats in associations with biofilm communities and intracellularly within a variety of protozoan hosts. Also the legionellae can survive in co-culture with various amoebae or with *Tetrahymena pyriformis* (8). Many of these studies also show that *L pneumophila* enhances the growth of amoebae, suggesting that the bacteria may provide some benefit. A similar symbiotic relationship has been studied with bacteria that naturally co-exist within amoebae. These bacteria, or X-bacteria, have an absolute requirement for their host, yet recent studies suggest that these organisms are related to the legionellae (77). X-bacteria are also essential for the infected amoebae hosts, in that curing of the bacterial infection with antibiotics leads to death of the hosts. It is not known whether the legionellae in natural environments exist as relatively benign symbionts in their natural hosts. *L pneumophila* may be more aggressive in macrophages than in nonlymphoid-derived cell lines and natural hosts (53). In this regard, high multiplicities of infection in the macrophage model result in the destruction of the monolayer (78), whereas, high multiplicities of infection have no effect on L929 cells or HeLa cells, even at multiplicities as high as 10,000 bacteria per eucaryotic cell (79). Fernandez et al (53) also noted that *L pneumophila* appeared to synthesize more Hsp60 in response to the intracellular milieu of the macrophage than when intracellular in L929 and HeLa cells. Perhaps in natural hosts, *L pneumophila* and related species embark on a mutualistic existence that establishes the longer term infection. *L pneumophila* bacteria released from a lysed host cell are motile for 24 h and within this time frame must find a suitable host (80). Therefore, it may be to the bacterium's survival strategy not to destroy the host too quickly.

In HeLa cells, the bacteria-laden phagosome persists long after the nucleus and other organelles within the cell have disintegrated (unpublished data). Moreover, this vacuole excludes trypan blue, indicating that the membrane remains energized and intact. In the absence of mitochondria, one can only speculate that perhaps the bacteria contribute to maintenance of the phagosome membrane. Perhaps Hsp60, which exhibits both ATPase and phosphotransferase activities, might in some way be involved because the protein associates with the phagosome membrane (53). Similar observations have been noted for the endosymbionts of various aphids (81,82). These endosymbiotic bacteria produce a major 60 kDa protein named symbionin that is synonymous with GroEL or Hsp60 (81). In one aphid system, bacterial secretion of Hsp60 is essential for a potato virus to complete its life cycle (83). There may be parallels to *L pneumophila* infection of eucaryotic hosts in which secretion of Hsp60 might function as a mediator, perhaps in conjunction with host chaperonins, to provide a buffer between host and parasite. Clearly, these intriguing possibilities warrant further investigation.

IMMUNITY AND A POSSIBLE CATCH-22

One must admit that the genus *Legionella* is highly successful in the natural environment and adapts well to man-made environments, including evaporative condensers, hot water systems, hot tubs, humidifiers and shower heads (22). Interestingly, several species of *Legionella* were the first to colonize waters surrounding Mount St Helen's following the volcanic eruption that sterilized the landscape. One might ask why a genus that is so ubiquitous in nature is not responsible for more disease. In answering this question, one must recognize that *Legionella* species did not evolve with humans; therefore, there has been no natural selection of virulence traits that would enhance survival in humans. In the lung of a mammal, the behavior of a macrophage probably is not much different from that of an aquatic amoeba. Humans with normal immune systems and few risk factors (3) are rarely infected. The author proposes that the very proteins that are secreted by *L pneumophila* early in the course of infection and are necessary for successful intracellular infection (ie, Hsp60), may be the key signatures of microbial infection that the immune system has evolved to recognize. It has been demonstrated that the lymphocytes from humans with acute legionellosis proliferate when challenged with pure *L pneumophila* Hsp60 (84). In addition, the Hsp60 class of proteins, including the Hsp60 of *L pneumophila*, specifically induce

expression of IL-1β and IL-12, that are key modulators of the host immune response (85-87). These cytokines stimulate natural killer cells, αβT cells and γδT cells to produce interferon-gamma (86,88). Interferon-gamma plays a critical role in the eradication of *L pneumophila* infection by activating macrophages to retard the intracellular multiplication of bacteria (62). Immunocompromised patients with suppressed cellular immune systems (ie, patients receiving cyclosporin A) are at much higher risk of succumbing to this infectious agent. In this regard, cardiac transplant patients are particularly susceptible to legionellosis (5). The human immune system has the capacity to target and destroy macrophages infected with an organism the immune system has never seen. Further study of how the immune system recognizes intracellular pathogens, using *L pneumophila* as a model system, might have important benefits for understanding and developing effective vaccine and antimicrobial measures against other intracellular parasites of humans including *Mycobacterium tuberculosis*, *Chlamydia trachomatis*, *Leshmania, Toxoplasma* and *Listeria* species, and many others.

SUMMARY

The past 20 years have seen the emergence of new microbial diseases, the re-emergence of others including streptococcal necrotizing faciitis and pneumococcal pneumonia, and the era of multiple-antibiotic resistance. *L pneumophila* will always be around because there is no realistic strategy to eradicate this organism from natural, and in some cases, man-made environments. The ability to diagnose infection as well as to distance susceptible individuals from known sources of legionella will continue to be the most effective strategies for prevention of infection. The bacteria themselves are remarkable in their host range and ability to deploy a simple strategy to subvert normal endocytic mechanisms in most eucaryotic hosts. In many ways, *L pneumophila* has become a useful tool for investigating many fundamental cell biology questions including organelle trafficking and signal transduction pathways. *L pneumophila* really is not the 'Monster Killer' that headlined the front pages of major newspapers in the summer of 1976. Instead, it is a rather seductive microbe that has found and exploited an Achilles heal of eucaryotic cells.

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