Editorial
Towards re-purposing BH3-mimetics in *Legionella* and viral infections

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In 1977, 29 of 182 patients who had attended an American Legion Meeting died due to lung infections. The then new disease was subsequently named Legionnaires' disease [1]. Infected individuals develop a range of symptoms whereby the most serious one resembles other bacterial lung infections that cause atypical pneumonia, resulting in pulmonary infiltrates and consolidation of lung tissue [2]. In the last decades, there has been an increasing trend of outbreaks and reported cases around the globe. Legionnaire's disease remains under-diagnosed and underreported and may thus account for more than 2–9% of all community-acquired pneumonia cases [2].

The Centers of Disease Control and Prevention reported that over 60% of all drinking water-associated outbreaks in the USA were due to *Legionella* in 2011–2012 [3]. This is because *Legionella* thrives in water cooling towers and distribution systems.

*Legionella* spp. are gram-negative bacteria that are ubiquitous in the environment. Of the 58 different *Legionella* species, 30 cause disease in humans. *Legionella pneumophila* serogroup 1 is the most common isolate from individuals with Legionnaires' disease, although current clinical tests are largely restricted to this serogroup [2]. Especially in Australia and Asia, *L. longbeachae* can account for up to 50% of all Legionnaires' diseases [4]. While *L. pneumophila* is found within man-made aquatic systems, *L. longbeachae* occurs primarily in garden soils and compost. Inhalation of contaminated aerosols, and presumably dust, is the most common route of infection, but a person-to-person transmission has recently been reported [5]. Humans are considered accidental hosts of *Legionella*. Within the environment, these bacteria replicate in single-cell protozoa and ameba. The intracellular survival of *Legionella* in ameba depends on a complex secretion system, termed Dot/Icm or Type IVB Secretion System (T4BSS), that transports ~300 effector proteins (~10% of the genome) into the cytoplasm of host cells [6]. In animal infection models, *Legionella* employs the very same secretion system to subvert the antimicrobial functions of lung macrophages. This reflects the conservation of molecular mechanisms that allow immune cells and ameba to digest bacteria. The T4BSS is absolutely required to establish infections in cultured ameba and macrophages and in lungs of susceptible mice [6]. This is largely because its effectors enable *Legionella* to establish a unique vacuole within ameba and macrophages that promotes bacterial growth [7]. Repeated rounds of intracellular growth, escape, and reinfections of new macrophages are thought to eventually overwhelm the host, such as susceptible mice. Immune responses that target *Legionella* directly or its protective vacuole restrict bacterial replication in lungs of infected mice [8]. It has also become apparent that *Legionella* may trigger immune reactions that further exacerbate disease [9]. For instance, human and mice infections are typically associated with high levels of inflammatory cytokines and immune cells, such as neutrophils, that may further augment the pathological symptoms associated with Legionnaire's disease [10,11].

Antibiotics, such as azithromycin, levofloxacin, and fluoroquinolones, are effective in killing all *Legionella* species tested in culture, and there have been no confirmed reports of acquired resistance in the clinic so far [2]. Despite state-of-the-art treatment, however, *Legionella* outbreaks typically cause fatalities in ~10% of infected individuals. In the most vulnerable patients, including the elderly, immunocompromised, and infants, the mortality rates remain high (>20%) [4]. In some cases, antibiotic treatment may fail to effectively eliminate *L. pneumophila*. This is due to inherent antibiotic resistance in the case of β-lactams and aminoglycosides. In addition, antibiotics may be ineffective in targeting intracellular *Legionella* due to reduced drug penetration and activity and/or changes in bacterial metabolism [12]. T4BSS effectors that function within the macrophage cytosol are thus attractive drug targets in preventing intracellular *Legionella* growth, but the functional redundancy among the 300 effectors has hampered the development of such compounds so far [13]. As an alternative approach, recent studies have identified host-directed compounds that show efficacy in preventing intracellular bacterial growth in cultured macrophages [14,15]. These compounds inhibit host factors that are hijacked by *Legionella* for intracellular replication. Whether this translates into effective therapeutic approaches against Legionnaire's disease awaits further validation.

*Legionella* must ensure the survival of its host cell for extended periods of time (>10 h) to enable sufficient bacterial growth. This is not an easy feat for an accidental pathogen that unlikely has evolved evasion strategies to prevent the activation of cellular suicide programs in macrophages that are typically absent in ameba. Indeed, *L. pneumophila* readily induces an inflammatory
form of macrophage death, termed pyroptosis, in mouse macrophages, which abrogates bacterial replication and triggers immune attack in mouse lung infections [9]. In addition, several L. pneumophila T4BSS effectors induce apoptosis, the major programmed cell death modality, by directly targeting mitochondria in ex vivo infections [16]. Mitochondria are known to generate reactive oxygen species to control invading microbes, and as such damaging these organelles may promote Legionella growth in ameba. It remains unclear, however, how inducing apoptosis in macrophages promotes lung infections. It is possible that host cell death due to damaged mitochondria also enables L. pneumophila escape whereby apoptosis merely accelerates the process of bacterial release. To control apoptosis in a timely manner, SidF was initially identified to inhibit pro-death BCL-2 family members in macrophage infections [17]. However, the effector is dispensable for infections in mice and macrophages and loss of SidF does not cause increased apoptotic macrophage death [18], suggesting that other mechanisms are at play. This may depend on L. pneumophila to cause the upregulation of pro-survival host factors, such as BCL-2, which are thought to extend macrophage viability until bacterial replication is completed, although this has not been formerly tested in lung infections [19,20].

The upregulation of BCL-2 family members has been reported in several experimental infections but has been extensively studied in cancers. Consequently, cancer research over the last 30 years has resulted in the development of small-molecule compounds that mimic the action of the pro-death BCL-2 family members, termed BH3-only proteins, and are thus commonly referred to as BH3-mimetics [21]. In susceptible cancers, BH3-mimetics induce rapid cancer cell death. One of these BH3-mimetics, venetoclax (ABT-199), targets BCL-2 with high affinity and has recently been approved in the USA and Australia for the treatment of some forms of leukemia [22]. Venetoclax and similar BH3-mimetic compounds are currently in more than 20 clinical trials to target other forms of cancers. Whether similar approaches may be of therapeutic value in infectious diseases is only just being investigated. For instance, a recent study has documented that BH3-mimetics are effective in preventing Legionella lung infection in mice [23]. Surprisingly, however, the BCL-2 targeting compound ABT-199 failed to abrogate Legionella infections in susceptible mice. Rather, the related pro-survival member, BCL-XL, was proven to be the Achilles heel of Legionella-infected mouse macrophages [23]. In the absence of BCL-XL, Legionella-infected macrophages induce mitochondria-mediated apoptosis, which prevented bacterial replication. Consistent with the genetic data, pharmacological inhibition of BCL-XL with navitoclax (ABT-263) or BCL-XL-specific BH3-mimetics induced apoptosis and impaired bacterial growth in cultured macrophages and prevented L. pneumophila and L. longbeachae lung infections in animal models [23]. This suggests that inducing apoptosis is effective in preventing bacterial replication and to trigger bacterial demise. The latter is likely independent of additional immune attack, as BH3-mimetic administration did not cause increased influx of inflammatory neutrophils and monocytes into infected mouse lungs. Rather, inducing apoptosis likely prevents excessive inflammation associated with Legionella infections, as the levels of the major inflammatory cytokines remained low in BCL-XL-deficient mice [23]. Consistent with this, overexpression of BCL-2 in macrophages and other innate immune cells leads to increased production of inflammatory cytokines in L. pneumophila-infected mouse lungs [24].

While pro-survival BCL-2 family members are essential for development and adult health, BH3-mimetic compounds are surprisingly well tolerated in mice [21]. This may be due to additional roles of BCL-2 family members beyond inhibiting apoptosis. Transient inhibition of BCL-2 family members with BH3-mimetics may also be insufficient to trigger adverse health effects. In addition, loss of a single BCL-2 family member does not necessarily trigger apoptosis. For instance, loss of BCL-XL did not cause apoptosis of uninfected macrophages, but killed infected cells [23]. This is because Legionella triggers the depletion of the BCL-XL-related protein, MCL-1. Several T4BSS effectors inhibit host protein synthesis and thus cause the rapid loss of MCL-1, a highly unstable protein required to maintain host cell viability under certain conditions [25]. Consequently, BH3-mimetics only kill mouse macrophages containing virulent Legionella and spare uninfected immune cells in mouse lung infections.

Many more pathogens inhibit host protein translation, suggesting that BH3-mimetic compounds may be effective in other infectious diseases [26]. Pseudomonas aeruginosa secretes the exotoxin A to block host protein synthesis, and inhibition of BCL-XL triggers rapid macrophage death after exotoxin A treatment [27]. Whether BH3-mimetic treatment is beneficial in Pseudomonas lung infections awaits to be seen. In addition to bacterial pathogens, viruses are well known to commandeer host protein synthesis to ensure sufficient translation of their own proteins. Viruses also frequently target BCL-2 family members to prevent apoptosis. Recently, infections with vaccinia and murine cytomegalovirus have been shown to trigger the loss of MCL-1 without inducing apoptosis. Macrophage cell death was only triggered after genetic deletion of BCL-XL or its inhibition with the BH3-mimetic compound ABT-737 [28]. This suggests that while some BCL-2 proteins are reduced during infections, inducing apoptosis requires inhibition of additional pro-survival proteins with BH3-mimetics. As observed in Legionella infections, targeting BCL-XL prevents viral replication in cultured macrophages. Whether this translates into an antiviral treatment in animals remains to be evaluated. It is tempting to speculate that other viruses that prevent host protein translation may be similarly susceptible to BH3-mimetic treatment. As such, targeting BCL-XL may well be a new frontier in anti-infective therapy research. This is particularly exciting as BH3-mimetic treatment is not affected by antimicrobial resistance. Given the looming threat of superbugs, killing infected cells with BH3-mimetic compounds promises an alternative and, likely additional, strategy in treating infectious diseases.

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References

Papers of special note have been highlighted as either of interest (+) or of considerable interest (+++) to readers.


• Identification of host-derived metabolites and immune responses that target intracellular Legionella.


• Together with reference 15 identified compounds that prevent intracellular Legionella replication by targeting host factors.


• Rational reengineering of a BH3-mimetic to target BCL-2 in clinical cancers.


