OLD DOMINION
UNIVERSITY
College of Science

To:
Morgan McGee, PhD
Current Pathogenesis Journal
456 Salk Hall
Norfolk, VA 23529

March 26, 2023

Dear Editor,

I would like to submit this manuscript "*Review of Legionella pneumophila*" for publication as an updated literature review in the journal *Current Pathogenesis.* This manuscript provides an overview of *Legionella pneumophila* discovery and pathogenesis, along with host response and treatments that have been researched and reported in the last 14 years. This review should be considered for publication as it contains relevant research on Legionella pathogenesis as well as research on eradication efforts. This submitted manuscript is an original literature work and has not been submitted for publication in other journals.

With community-acquired pneumonias being a leading cause of infection and death worldwide, having current research on the pathogenesis of a microbe such as *L. pneumophila* helps further understanding and eradication efforts. As for community-acquired pneumonias, *L. pneumophila* is considered more atypical and is less understood than other pneumonia types such as *Streptococcus pneumoniae;* of the thirty-three primary and review articles studied for this manuscript, twenty-three articles met needed criteria for providing current information on this microbe. This updated manuscript will provide the public and other researchers with imperative information on this microbe including recent studies that are sure to spark new propositions for *L. pneumophila* eradication.

Thank you for your consideration in this piece.

Sincerely,

Madison Moody
Undergraduate
Department of Biological Sciences
123 Pasteur Way
Norfolk, VA 23529

Dear Dr. BugerShoulder,

I would like to thank the editor and two reviewers for their comments as well as the chance to revise and resubmit this manuscript. I have made changes as needed based on reviewer comments. Each revision was taken into consideration for this review and an explanation for each reviewer comment has been responded to below.
****

Professor: Dr, BurgerShoulder
Comments to the Author

Please use subheadings. Does not read or flow well.

1. [Our response]: Subheadings have been appropriately added to this manuscript to allow for a better flow and clearer message.

What is line 42 saying? Word choice (like "suited with") is confusing/distracting.

2. [Our response]: The original line 42 stated “Once this bacterium is in our lungs and initiates strategic contact with alveolar and mucosal macrophages via phagocytosis, the bacteria are able to being their process in pathogenesis.” This line has been reviewed to give a clearer idea of the bacteria’s goal. Revised to, “Once this bacterium enters our lungs, it initiates strategic contact with alveolar and mucosal macrophages to being its pathogenic process.” The DUKE assignment was also used to revise sentences for better word choice and syntax.

Referee: 1
Comments to the Author

There are no headings to separate one main idea section from the next. Make sure that you use similar wording as stated in the instructions for what Dr. S wanted you to talk about. For example, there were a total of four (Microorganism Biology and Lifestyle, Pathogenesis and Host Reponses, Control and Eradication, Future Challenges and Opportunities), including a discussion/conclusions section.

1. [Our response]: Subheadings have been appropriately added to this manuscript to allow for a better flow and clearer message. I agree with this revision; although it is not stated in the rubric to have these subheadings providing them through the paper helps clarify what it being discussed. A discussion/conclusion section was not added as a subheading.

When you have a figure/table in a scientific paper, it is expected that it is first addressed in the paragraph before the figure/table, to be viewed by the reader. For example, the specific section in the paragraph would state “In Figure 1...” or “…, as illustrated in Figure 1.” Also be sure to be consistent with the figure legend starter. It would be best to start your figure legends of as Figure 1. and Figure 2. and Figure 3., instead of Figure 1., Figure 2:, and Figure 3..

2. [Our response]: Having consistent formatting of figure placement is important for the flow of a review article. With this revision, all three figures have been replaced to present to the reader after the subject is mentioned in the paragraph preceding the figure. Consistent formatting for all figure legend starters was also applied.

What is cryoFIB, cryoSEM, and cryoET? This is asked because the words were briefly stated, with no explanation of what the words were.

3. [Our response]: In writing this review, it was assumed that readers would understand some laboratory methods without having to explain them. The methods are not as important for this review manuscript as the results are. However, I agree that adding in a short description will provide insight to the methods used. Including a brief mention of cryoFIB, cryoSEM, and cryoET being forms of electron microscopy and cryotomography has been revised to this manuscript.

Overall, it is suggested that you use a grammar checker and/or carefully read through to make sure that sentences flow as you would like for them to and as they should, based on what is stated.

4. [Our response]: This comment is not very specific or beneficial. After this manuscript draft was turned in a DUKE assignment was completed that gave specific examples of things to look for that helped better the paper. This comment lacks productive feedback, therefore, no changes were made based on this suggestion.

Referee: 2
Comments to the Author

Some paragraphs are missing citations

5. [Our response]: This comment is correct but not specific. It is imperative that all information used in a review is accredited to the source that information is taken from. In this manuscript citations were located at the end of a complete thought rather than at the end of each sentence. This suggestion made was considered and every sentence of the final assignment is cited when information was taken from a source. A more useful comment would include the line numbers in which the reviewer is referring to.

Ends of paragraphs should have citations as these are not your own thoughts. Citations should be made before punctuation, Ex: ".....[9]." Line 149: needs citation at end. Line 153: needs citation at end.

6. [Our response]: I agree with this feedback. It is appreciated that line numbers were included in reference to what changes are suggested to be made, giving an example of what the reviewer means. Revisions to the entire paper were made placing the citation of each sentence before the punctuation. Sentences that were not my own thoughts were cited as well. Each applicable sentence has a citation in this manuscript.

Line 33: "during this time was conclusive..". Was this meant to say "wasn't conclusive"?

7. [Our response]: Changed as suggested.

Line 146-147: "From this its supported…" need a comma after "this" and "its" should be "it's"

8. [Our response]: Changed as suggested.

Line 196-197: "...proinflammatory cytokines are pairs with anti-inflammatory.." replace "are pairs" with just "pair".

9. [Our response]: Changed as suggested.

Review of *Legionella pneumophila*

Abstract: *Legionella pneumophila* is a gram-negative bacillus pathogen that is found living in public water systems and intracellularly in host alveolar macrophages. After the 1976 American Legion convention outbreak in Philadelphia, *L. pneumophila* posed a great threat to the human population, presenting similarly to other community acquired pneumonias. This review article highlights the pathogenesis and host response to *L. pneumophila,* including treatment and plans for controlling the disease. Primary and review articles referenced were all published on the PubMed website; a total of 23 were referenced in this review. Primary research from the last decade observes secretion systems, T4SS and T2SS, which have given insight into how *L. pneumophila* is a virulent threat to host alveolar macrophages. In tandem with T2SS, legionella containing vacuoles, LCV, disrupt proper host Golgi function as endoplasmic reticulum associated proteins surround the LCV. Disruption of normal host function such as impairment of phagocytosis poses a threat to host prognosis. The bacteria's ability to evade host response such as inhibiting TLR-5 functions by cleavage of bacterial flagellin allows for more replication in hosts which leads to septic shock. Research regarding host response mechanisms includes sufficient IL-1 induction and specific proteins that decrease pulmonary inflammation. Traditional antibiotic treatment plans include fluoroquinolones or macrolides to clear infection, however a recent study using glycylcline for disease treatment of an immunocompromised patient sheds light on alternative control plans. Findings reported in this review article are relevant to *L. pneumophila* and are informative on pathogenesis characteristics, host responses, and treatment plans. With many of the referenced articles being primary, they have good internal validity. However, it would be appropriate to say repeating methods multiple times would give greater validity to information regarding all findings and treatment plans.

Biology and Lifestyle

Legionella pneumophila is a type of gram-negative bacillus bacteria that can live in water systems and intracellularly in a variety of hosts (1). This microorganism received its name, *Legionella pneumonia,* after a 1976 outbreak at the annual convention of American Legion in Philadelphia; a non-profit organization that includes veterans of the United States (2). The source of transmission in this outbreak was traced back to the hotels cooling system which supported bacterial growth on biofilms (2). Of the 182 Legionnaires' that contracted the disease 29 did not survive the infection (2). Following the outbreak and identification of a possible life-threatening bacteria, it has been a mission to better understand the characteristics and capacity that *L. pneumophila* has (2). This opportunistic bacteria *L. pneumophila* overtakes human macrophages resulting in Legionnaires' disease or in milder cases, Pontiac fever (1). The disease is categorized by characteristics very similar to other forms of pneumonia (3). In addition to being gram-negative, this bacterium is pleomorphic and can morph from coccoid and rod shapes throughout its life span (1). These microbes have flagellum that allows them to be mobile in host and outside environments, some are monopolar, but others can present with two or three polar or lateral flagella (1).

Legionella pneumophila is broken into fifteen different serogroups with *L. pneumophila* serogroup 1 being the causative agent for approximately 90% of all Legionnaire's disease (1). Serogroups 2-15 have been identified in only 15 – 20% of *L. pneumophila* community-acquired pneumonias (1).

In nature, *L. pneumophila* typically exists in aquatic environments and is seen to be a threat when forming biofilms on water sources that our population accesses such as lakes, air conditioning units, cooling towers, fountains, and spas (1). These locations are optimal for *L. pneumophila,* since the bacteria is most viable in temperatures ranging from 22.3-43.5C (1, 2). Research that shows there are greater *L. pneumophila* incidences in water sources that are less chlorinated along with pulmonary environments (2). This research supports *L, pneumophila* preferring areas of a more neutral pH ranging from 5.0-8.5 (2). The bacteria survive intracellularly in amoeba and humans relying on the tricarboxylic acid cycle for aerobic respiration (3).

Pathogenesis

To better understand the causation of these casualties, lung tissues of the dead Legionnaire's patients was collected for research, inoculating rodents with the dead tissue (4). The bacteria observed during this time was not conclusive of anything and it was not until months later that microbiologist Joseph McDade was able to grow *L. pneumophila* in embryonated eggs and from this isolate a pure *L. pneumophila* culture (4, 5).

Legionella pneumophila is transmitted into human hosts by aerosolized droplets that are formed from the water sources accessed by our population (2-5). When aerosolized droplets containing *L. pneumophila* are inhaled, the bacteria are transported from the environment, through human airways, and into the pulmonary tract (2-5). Here in the lower respiratory tract, involving the lungs, the bacteria can nest in lung alveoli (3). Once this bacterium enters the lungs, it initiates strategic contact with alveolar and mucosal macrophages to being its pathogenic process (3).

For intracellular survival, bacteria must have access to the cell host cytosol and mitochondria to avoid lysis (6). *Legionella pneumophila* is seen to use two secretion systems, specific amylases, and macrophage infectivity potentiator proteins to accomplish this goal (6-13). The two different secretion system types, Type II and IV Secretion Systems take control of host cell function, using available space and nutrients for further provocation (6). Secretion systems, T4SS and T2SS, rely on each other for optimal protein translocation (7). Translocation and secretion in host cytosol by *L. pneumophila* involves a key defense mechanism that creates a "safe space" for *L. pneumophila* known as Legionella Containing Vacuoles, *Figure 1* (6). Legionella vacuoles act as a membrane bound structure that avoids the fusion of bacteria vacuole and phagosome with the host lysosome (6, 7).

T4SS is a major virulence factor for *L. pneumophila* consisting of multiprotein complexes *(6, 8, 9).* Type 4b secretion system, T4bSS, is essential for intracellular replication as it inserts virulent effector proteins into the host cytoplasm (6). The function of T4SS is to help mature the LCV and recruit the endoplasmic reticulum for translocation (6). This can be seen in a laboratory setting by infecting *Acanthamoeba castellanii* with *L. pneumophila* (6). In turn, the ER attaches its cell pole to the Legionella containing vacuole membrane, observed using cryoFIB, cryoSEM, and cryoET; forms of

electron microscopy and cryotomography (1, 6). Doing this allows for *L. pneumophila* to fuse with the mitochondria and recruit the endoplasmic reticulum exit vesicles to form around the outside of the legionella containing vacuole (6). This formation around the LCV appears to be similar to the structure of the rough ER in the host cell (7). Type 2 secretion system, T2SS, also translocate proteins but into the extracellular environment. The ability to use secretion systems for intracellular replication makes *L. pneumophila* more of a threat (7).

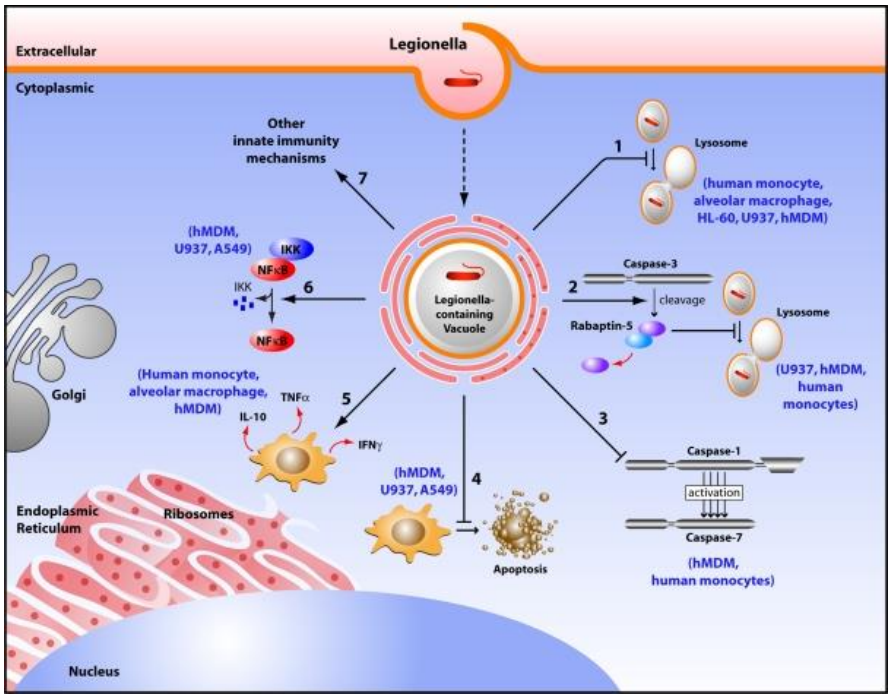

Figure 1. *Legionella Mechanisms. This figure represents Legionella going from an extracellular environment into an intracellular environment. To survive. Legionella form a vacuole that allows for the avoidance from fusion with host cell lysosome. From the safety of the legionella containing vacuole, different defense mechanisms and effector proteins can aid the bacteria in replication. Copied from (7).*

Type 2 Secretion System has peptides such as Pseudomonas aeruginosa peptidase which allows proper Type 2 Secretion (7). Type 4 Secretion System is reliant on the peptide from Type 2 Secretion System for pilus formation (7). Translocation is of great benefit for *L. pneumophila,* allowing the cross from the epithelium of the lungs to other vital organs (2, 7). This migration can be seen over time if not treated leading to septic shock (2). With over 300 effector proteins being inserted into host macrophages, translocation not only allows for the spread of the bacteria, but it evades immune defense by inhibiting eukaryotic protein synthesis (2, 6-8, 10).

With T4SS being a factor in *L. pneumophila*, it's important to observe the function of T4SS is along with T4SS inhibitors (3, 11). High-throughput screening for compounds that attenuate reporter delivery by T4SS in *L. pneumophila* identified by FRET-based translocation assay showed inhibition to the T4SS system without effecting host functions such as replication and phagocytosis (11). This was accomplished by carrying out quantitative high throughput screening at different concentrations fused

with β-lactamase-LidA protein to observe interference with T4SS-mediated translocation (3, 11).

In addition to secretion systems, specific amylases and macrophage infectivity potentiator proteins are essential for intracellular replication (8). LamB amylase has an alpha-amylase domain and a conserved catalytic cite that is structurally similar to other crystalized glucosidases seen using the I-TASSER database (8). With *L. pneumophila* accessing the host cytosol by translocation of proteins in T4SS or T2SS, LamB amylase is a possible substrate for this bacterium (8). In T2SS, LamB was not found to relate to this type of secretion system as it is missing an N-terminal that is a characteristic of T2SS substrates (8). Using adenylate cyclase report functions, the C-terminus in LamB was identified in seventeen of the one hundred amino acids that are C-terminus effector proteins of T4SS, which aid in replication (8).

Commonly seen in T2SS is the bacteria located inside of a vacuole, referred to as Legionella containing vacuole, LCV (6). Here the bacteria are able to resist lysosomal fusion protecting the bacteria from lysis. Inside the cell, endoplasmic reticulum associated proteins locate themselves around the legionella containing vacuole (6, 12). The typical job of the endoplasmic reticulum associated proteins, or ribosomes, is to bring messages to the Golgi body for further maturation and storage for cellular function (12). When ER proteins associate with the LCV, interference with normal host Golgi function is observed (12). Ubiquitin regulator effector proteins allow for target proteins to reach desired destinations (12). This is accomplished by a covalent bond between the C-terminal of a carbonyl group and the amine residue of a target protein (12). Ubiquitin signaling is an essential part for cellular functioning, allowing for the identification, tagging, and cleaving of unwanted proteins (12). Once inside a host cell, *L. pneumophila* effector proteins can regulate host ubiquitin signaling because their genome encodes for proteins that are similar to eukaryotic ubiquitin ligases (7, 10, 12).

In an investigation of RavA, which is acknowledged to be a type of *L. pneumophila* effector, it's seen that the C-terminal segmentis used to localize the Golgi apparatus when cells are ectopically expressed (3). By transfecting HEK293T cells with either green florescent protein or RavA-GFP, the findings of Golgi localization by RavA was supported (3). With *L. pneumophila* LCV protected by ER studded with ribosomes, *L. pneumophila* can replicate using its translocated effector proteins (3).

In studying the pathogenic effects that LamB amylase has on intrapulmonary growth with *L. pneumophila* in *vivo*, researchers injected LamB into mice, observing them for ten days (8). Results from this study show that fifty percent of the mice with only the wild type *L. pneumophila* all died within the ten day span, while all of the LamB treated mice were alive at the conclusion of the ten days, *Figure 2 (8)*. With knowledge that *L. pneumophila* lives off of amino acids as a fuel source for the tricarboxylic acid cycle LamB, a structure similar to other crystalized glycosidases, LamB amylase, acting as a nutritional source, is significant in intracellular growth in human macrophages but is not confirmed to translocate proteins in the T4SS (8).

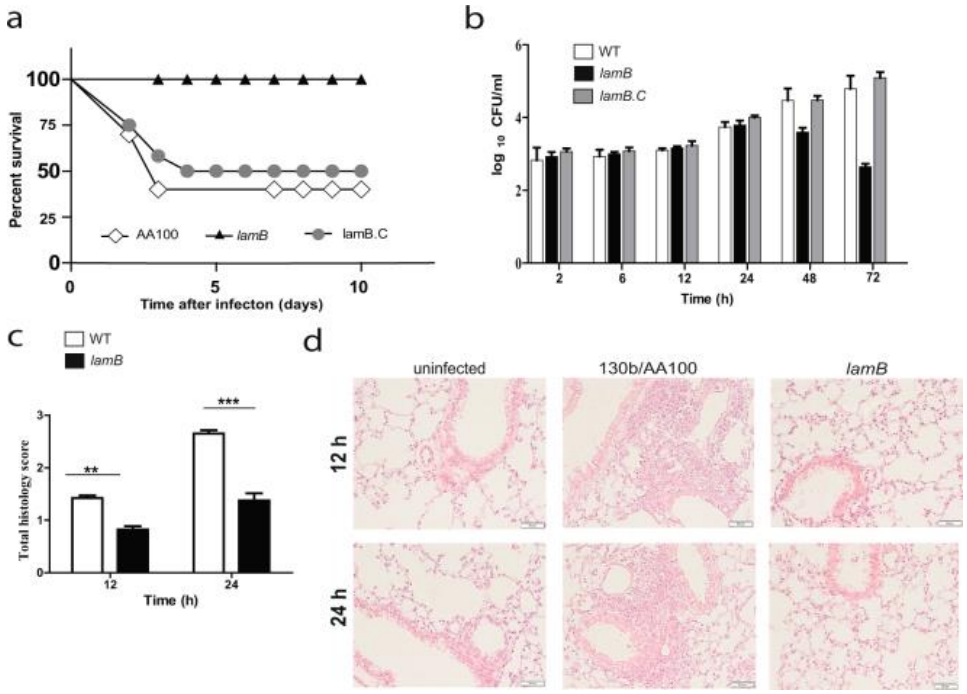

Figure 2. *Legionella pneumophila amylase study. Amylase is essential for intracellular replication in human macrophages and amoebae study. This figure represents the study done to analyze the possible role of LamB amylase in cases of Legionella pneumophila. It is seen in section (A) that after 10 days of infection the mice transfected with LamB amylase survived compared to the wild type mice (AA100) and the LamB.C mice. Section (B) illustrates the results of colony forming units in each of the three transfected mouse types. After 72 hours the LamB transfected mice had a significantly lower number of colony forming units compared to the other two mice types. Section (C) results show a lower histology score for LamB mice and section (D) provides histology slides of the lung tissues observed from uninfected, wild type 130b/AA100, and LamB mice after a 12 and 24 hour period. Copied from (8).*

Aiding in *L. pneumophila* virulence is its ability to escape death by using macrophage infectivity potentiator proteins, MIP proteins, when phagocytized by alveolar macrophages (13). To investigate the influence of MIP proteins on phagocytosis and chemotaxis of RAW264.7 macrophages, specific MIP protein components of *L. pneumophila* correlated with Legionella survival (13). Cells cultured with MIP showed expression of lncRNA GAS5 and microRNA miR-21 resulting in a reduction in phagocytosis and an enhancement in chemotaxis (13). LcnRNA GAS5 has the ability to effect the ability of interfere with miRNA hindering the regulatory effect it has on mRNA (14). The mechanism of macrophage infectivity potentiator proteins, MIP, is not yet understood but the results from RAW264.7 macrophage interactions prove MIP to be detected in *L. pneumophila* infection (13). The greater dose of MIP into RAW264.7 cells resulted in a trend of an increased chemotactic index (13). From this it's supported that macrophage infectivity potentiator proteins can impair phagocytosis in macrophages infected by *L. pneumophila* advancing the growth of the bacteria in host environments (13).

Host Response

The clearance of an opportunistic infection is important for host health. For the body to start signaling for the clearance of this pathogen it must first be able to recognize the foreign pathogen (15). The T4SS expression previously mentioned is one of the first signals to the body that something abnormal is occurring in *vivo* (15). Neutrophil recruitment is a result of the expression of bacterial secretions from Dot/Icm T4SS (7, 15). Crucial toll like receptors are responsible for the initiation and activation of chemokine signaling cascades (15). Toll like receptors can recognize a wide range of pathogen associated molecular patterns, PAMPs (15). PAMPs allow for the initiation of cytokine response and clearance of *L. pneumophila (15).* Toll like receptors TLR2, TLR3, TLR4, TLR5, and TLR9 have been discovered as specific receptors that are crucial in the initiation of cytokine cascades for *L. pneumophila (7, 15, 16).* It is understood that *Legionella pneumophila* persists for a period of time intracellularly in vacuoles before lysing macrophages and moving onto the next. Having TLRs both of outside of a host cell and on the inside of a host cell gives hosts the best chance at identifying the pathogen (15). Toll like receptor -2, -4, -5 are found on the cell surface while TLR-3,-4,-9 are located on endosomal vesicles, TLR-4 occurs in both locations (15). These TLR bind to the *L. pneumophila* cell wall containing LPS as well as binding to motile characteristics such as the flagellin (16). Specifically, TLR 5 has been seen to effectively bind with Legionella flagellin to initiate an immune response by production of TNF-alpha (7, 16). Cytokine production of TNF-alpha and TNF-gamma is a part of the hosts innate immune system to activate antimicrobial signaling (7). In pathogenic efforts to evade this response *L. pneumophila* has adopted the ability to cleave their flagellin using zinc metalloprotease ProA which in turn acts as a TLR-5 inhibitor blocking any signaling cascade (16). The less viable TLRs are, the poorer the prognosis in Legionella clearance; without TRLs, the initiation of chemokine release such as IL chemokines is unable to take place (16).

To test the importance of type-1 IL-1 receptors in associated lung tissue, mice with IL-1R deficiencies and mice with sufficient IL-1R were inject with *L. pneumophila* to observe Legionella formations (17). Using flowcytometry, colony forming units were identified in both mouse types comparing bacteria from the bronchoalveolar lavage (17). This site of replication is similar to where the bacteria would be seen in human hosts (17). It is seen in mice that are deficient in type-I IL-1 receptors are more prone to contracting a *L. pneumophila* infection than those with the type-1 IL-1 receptors (17).

In addition to IL-1, IL-alpha and IL-beta are seen to work at different times during the recruitment of neutrophils to the lungs (17). In this research study, the mice injected with IL-1-alpha had detected activity after only three hours of infection while IL-1-beta activity started after six hours of infection (17). These findings support that in human immune response IL-1-alpha is essential to neutrophil recruitment in early IL-1R dependent and T4SS dependent recruitment (17). Something notable in this research that supports IL-1-alpha's role in host response is *L. pneumophila* that does not have effector proteins such as essential MIP or ubiquitin effector proteins is unable to block this response or induction of IL-1alpha (17).

The ability to induce IL-1-alpha increases the ability to clear infection (17). Hosts with the capacity to induce IL-1-alpha against *L. pneumophila* inhibitions will have a greater prognosis (17). This study shows in *vitro* and in *vivo* that despite translation inhibition from *L. pneumophila* defenses, TLR activation it is sufficient enough to induce

IL-1-alpha (17). By looking at the types of cytokines present during an infection researchers are able to predict whether an immune cell is responding to the site of infection as an active helper or a possible bacterial target (17) . Every humoral response requires balance, proinflammatory cytokines pair with anti-inflammatory cytokines (7). More of one type of cytokine is insightful for the type of interaction that is happening within a site of infection. For example, IL-10 is an anti-inflammatory signal that acts against the bactericidal activity of TNF-alpha and TNF-gamma (7). A production of IL-10 may be a virulence factor illuding that *L. pneumophila* is able to promote bacterial replication (7).

With immune responses initiating inflammation cascades, controlling inflammation can be improved by peptides such as Thymosin-β4 (18). The expression of certain peptides also aids in the host immune response for *L. pneumophila* defense. Thymosin-β peptides in synthesis can promote cell migration, angiogenesis, and differentiational tasks important in immune response (18). This peptide also defends against apoptosis and promotes repairment of damages tissue cells (18, 19). Support for this notion is shown from an increased resistance of *L. pneumophila* in mice during pulmonary and systemic infection in mice. Mice transduced with T-β4 show an increased survival period and decreased pulmonary inflammation (18). It is understood in human immune systems that toll like receptor activity is correlated with the expression of Thymosin-β4. In this study with T-β4 transgenic mice, the number of cytokines expressed in T-β4-TG mice decreased the number of *L. pneumophila* able to survive in macrophages, placing a greater value on T-β4 expression being a key function in immune response, *Figure 3* (18). This remarkable response with T-β4 is also something to take note of in possible treatment plans for *L. pneumophila* (18).

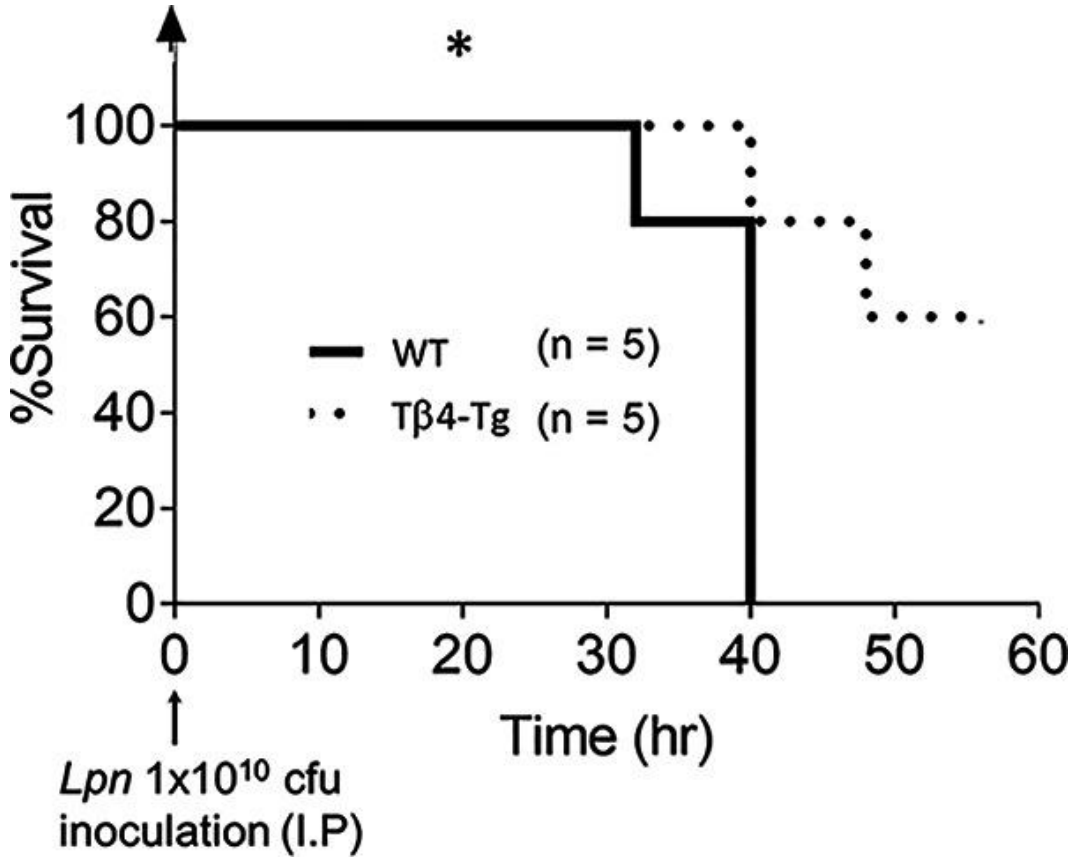

Figure 3. *Transgenic mice survival. This figure represents the data collected from a study where mice were treated with Thymosin-β4 which reflects the increased survival rate in mice that were injected with Legionella pneumophila-induced septic shock. It illustrates that the wild type mice were all dead by the 40-hour post inoculation time while the mice treated with transgenic thymosin 4 beta were able to live a prolonged life after inoculation of Legionella pneumophila. Copied from (18).*

Control and Eradication

Clinically in *L. pneumophila* infections, late identification can be detrimental for patients that are immunocompromised, geriatric, or have previous health conditions (20). *Legionella pneumophila* evades immune defenses surviving host macrophages. The first presentation of *L. pneumophila* infection presents much like symptoms of influenza (20). It is typical for one to experience a fever, chills, muscle pains, coughs, and even confusion (20). Though our bodies have incredible immune capabilities *L. pneumophila* cannot always be cleared by our immune defenses (20). Early identification of *L. pneumophila* allows treatment to intercept infection before it becomes systemic leading to death (20).

In hospital settings *L. pneumophila* can be identified in a variety of ways; common practices use urine sampling, sputum sampling, and blood sampling (20). Like most cases of community-acquired pneumonia, patients are prescribed antibiotics, sometimes in combination, to help cure infection (20-22). Treatment includes oral medication of antibiotics that are taken for multiple days to allow antibiotics to take

intended course (20-22). There is no optimal antibiotic treatment length established for this community acquired pneumonia; however, it is agreed upon for antibiotic use to persist for an additional five days after patient reaches clinical stability (20). Treatment length on average last 3-7 days for moderate cases and 10-14 for patients who are immunocompromised (20). Most common treatment plans include antibiotics that consist of fluoroquinolones or macrolides (21, 23). There is not large evidence for which type of antibiotic is more effective from lack of randomized control trials (23). In a meta-analysis of 3,525 patients, there was little difference in mortality rate between patients treated with fluoroquinolones versus macrolides at 6.9% and 7.4% respectively (20).

Fluoroquinolones such as levofloxacin and moxifloxacin, or macrolides including azithromycin and clarithromycin are the typical antibiotics included to treat any community-acquired pneumonia (20-23). Of reported US cases the median delay to starting antibiotics following pneumonia diagnosis was five days in patients who did not survive and a median delay of one day for those who survived (20). This delay in treatment is in addition to the delay in diagnosis, since most patients do not present symptoms for multiple days following the infection (20). Early identification and treatment of a patient with such pneumonia is imperative for the patients' health and recovery from such bacterial disease (20-23).

Future Challenges and Opportunities

Depending on severity of the case and length of infection, prescriptions from clinics may be different (21). For example, it is recommended for patients with *L. pneumophila* infections that are immunocompromised to use levofloxacin and azithromycin over moxifloxacin and clarithromycin (21). Challenges such as contraindications to fluoroquinolones or macrolides, along with possible heart arrythmias and health conditions restrict people from being quickly treated using these two antibiotic types (21). However, a recent Legionnaire's disease case study using tigecycline resulted in successful treatment of the disease (21). The 61-year-old immunocompromised Caucasian male was unable to accept either fluoroquinolones or macrolides from previous allergic reactions to the antibiotics (21). The male was administered tigecycline, a third generation glycylcline, as an alternative to preferred treatments (21). After a fourteen day treatment of tigecycline intravenously, the infection was cleared and no reoccurrence of infection was noted three months post treatment (21). This case study sheds light on possible future treatment methods for *L. pneumophila* especially with the increased resistance that bacteria species have to antibiotics now (21).

Taking a preventative approach to eradicate this disease would involve reducing the amount of *L. pneumophila* in common water sources that the public has access to (1). Current treatment plans for disinfecting water distribution systems includes thermal and chemical methods (1). The use of UV light, thermal shock, and various chlorination methods using chemicals such as monochloramine, chlorine dioxide, and hyper chlorination are all used in efforts to disinfect the systems that supply the population with water (1). Even with these preventions in place there are still recurrent places of *L. pneumophila* (1). The idea behind persistent *L. pneumophila* in water systems is their

ability to survive well in amoeba and various biofilms despite the chemical and thermal parameters that are in place (1, 2, 5).

Challenges that have been identified in eradicating *L. pneumophila* include patient restrictions, *L. pneumophila* antibiotic resistance, and biofilm or water distribution treatment systems (1, 2, 5). Eradication efforts that properly balance between eliminating *L. pneumophila* from public water systems and maintaining safe water for public use are still being researched for future works (5). Based on our current knowledge of antibiotic resistant microbes, looking at case studies of successful treatment plans opens new doors for alternative methods to treat *L. pneumophila* (1, 2, 5).

References

1. Knežević M, Rončević D, Vukić Lušić D, Mihelčić M, Kogoj R, Keše D, et al. Decreasing Pasteurization Treatment Efficiency against Amoeba-Grown Legionella pneumophila-Recognized Public Health Risk Factor. Int J Environ Res Public Health. 2022;19(3). Epub 20220119. doi: 10.3390/ijerph19031099. PubMed PMID: 35162120; PubMed Central PMCID: PMC8834526.
2. Iliadi V, Staykova J, Iliadis S, Konstantinidou I, Sivykh P, Romanidou G, et al. Legionella pneumophila: The Journey from the Environment to the Blood. J Clin Med. 2022;11(20). Epub 20221018. doi: 10.3390/jcm11206126. PubMed PMID: 36294446; PubMed Central PMCID: PMC9605555.
3. Chung IYW, Li L, Tyurin O, Gagarinova A, Wibawa R, Li P, et al. Structural and functional study of Legionella pneumophila effector RavA. Protein Sci. 2021;30(5):940-55. Epub 20210309. doi: 10.1002/pro.4057. PubMed PMID: 33660322; PubMed Central PMCID: PMC8040872.
4. McDade JE, Shepard CC, Fraser DW, Tsai TR, Redus MA, Dowdle WR. Legionnaires' disease: isolation of a bacterium and demonstration of its role in other respiratory disease. N Engl J Med. 1977;297(22):1197-203. doi: 10.1056/nejm197712012972202. PubMed PMID: 335245.
5. Winn WC, Jr. Legionnaires disease: historical perspective. Clin Microbiol Rev. 1988;1(1):60-81. doi: 10.1128/cmr.1.1.60. PubMed PMID: 3060246; PubMed Central PMCID: PMC358030.
6. Böck D, Hüsler D, Steiner B, Medeiros JM, Welin A, Radomska KA, et al. The Polar Legionella Icm/Dot T4SS Establishes Distinct Contact Sites with the Pathogen Vacuole Membrane. mBio. 2021;12(5):e0218021. Epub 20211012. doi: 10.1128/mBio.02180-21. PubMed PMID: 34634944; PubMed Central PMCID: PMC8510526.
7. Khweek AA, Amer A. Replication of Legionella Pneumophila in Human Cells: Why are We Susceptible? Front Microbiol. 2010;1:133. Epub 20101228. doi: 10.3389/fmicb.2010.00133. PubMed PMID: 21687775; PubMed Central PMCID: PMC3109522.
8. Best A, Price C, Ozanic M, Santic M, Jones S, Abu Kwaik Y. A Legionella pneumophila amylase is essential for intracellular replication in human macrophages and amoebae. Sci Rep. 2018;8(1):6340. Epub 20180420. doi: 10.1038/s41598-018-24724-1. PubMed PMID: 29679057; PubMed Central PMCID: PMC5910436.
9. Liu X, Shin S. Viewing Legionella pneumophila Pathogenesis through an Immunological Lens. J Mol Biol. 2019;431(21):4321-44. Epub 20190725. doi: 10.1016/j.jmb.2019.07.028. PubMed PMID: 31351897; PubMed Central PMCID: PMC9332137.
10. Jeong M, Jeon H, Shin D. Ubiquitin-regulating effector proteins from Legionella. BMB Rep. 2022;55(7):316-22. doi: 10.5483/BMBRep.2022.55.7.054. PubMed PMID: 35651329; PubMed Central PMCID: PMC9340081.
11. Cheng E, Dorjsuren D, Lehman S, Larson CL, Titus SA, Sun H, et al. A Comprehensive Phenotypic Screening Strategy to Identify Modulators of Cargo Translocation by the Bacterial Type IVB Secretion System. mBio. 2022;13(2):e0024022. Epub 20220308. doi: 10.1128/mbio.00240-22. PubMed PMID: 35258332; PubMed Central PMCID: PMC9040768.

12. Isberg RR, O'Connor TJ, Heidtman M. The Legionella pneumophila replication vacuole: making a cosy niche inside host cells. Nat Rev Microbiol. 2009;7(1):13-24. Epub 20081117. doi: 10.1038/nrmicro1967. PubMed PMID: 19011659; PubMed Central PMCID: PMC2631402.
13. Shen Y, Xu J, Zhi S, Wu W, Chen Y, Zhang Q, et al. MIP From Legionella pneumophila Influences the Phagocytosis and Chemotaxis of RAW264.7 Macrophages by Regulating the lncRNA GAS5/miR-21/SOCS6 Axis. Front Cell Infect Microbiol. 2022;12:810865. Epub 20220429. doi: 10.3389/fcimb.2022.810865. PubMed PMID: 35573783; PubMed Central PMCID: PMC9105720.
14. Paraskevopoulou MD, Hatzigeorgiou AG. Analyzing MiRNA-LncRNA Interactions. Methods Mol Biol. 2016;1402:271-86. doi: 10.1007/978-1-4939-3378-5_21. PubMed PMID: 26721498.
15. Grigoryeva LS, Cianciotto NP. Human macrophages utilize a wide range of pathogen recognition receptors to recognize Legionella pneumophila, including Toll-Like Receptor 4 engaging Legionella lipopolysaccharide and the Toll-like Receptor 3 nucleic-acid sensor. PLoS Pathog. 2021;17(7):e1009781. Epub 20210719. doi: 10.1371/journal.ppat.1009781. PubMed PMID: 34280250; PubMed Central PMCID: PMC8321404.
16. Scheithauer L, Thiem S, Ünal CM, Dellmann A, Steinert M. Zinc Metalloprotease ProA from Legionella pneumophila Inhibits the Pro-Inflammatory Host Response by Degradation of Bacterial Flagellin. Biomolecules. 2022;12(5). Epub 20220422. doi: 10.3390/biom12050624. PubMed PMID: 35625552; PubMed Central PMCID: PMC9138289.
17. Barry KC, Fontana MF, Portman JL, Dugan AS, Vance RE. IL-1α signaling initiates the inflammatory response to virulent Legionella pneumophila in vivo. J Immunol. 2013;190(12):6329-39. Epub 20130517. doi: 10.4049/jimmunol.1300100. PubMed PMID: 23686480; PubMed Central PMCID: PMC3682686.
18. Park B, Shin MH, Kim J, Park G, Ryu YK, Lee JW, et al. Ectopic Expression of Human Thymosin β4 Confers Resistance to Legionella pneumophila during Pulmonary and Systemic Infection in Mice. Infect Immun. 2021;89(4). Epub 20210317. doi: 10.1128/iai.00735-20. PubMed PMID: 33468581; PubMed Central PMCID: PMC8090946.
19. Crockford D, Turjman N, Allan C, Angel J. Thymosin beta4: structure, function, and biological properties supporting current and future clinical applications. Ann N Y Acad Sci. 2010;1194:179-89. doi: 10.1111/j.1749-6632.2010.05492.x. PubMed PMID: 20536467.
20. Viasus D, Gaia V, Manzur-Barbur C, Carratalà J. Legionnaires' Disease: Update on Diagnosis and Treatment. Infect Dis Ther. 2022;11(3):973-86. Epub 20220503. doi: 10.1007/s40121-022-00635-7. PubMed PMID: 35505000; PubMed Central PMCID: PMC9124264.
21. Arget M, Kosar J, Suen B, Peermohamed S. Successful Treatment of Legionnaires' Disease with Tigecycline in an Immunocompromised Man with a Legion of Antibiotic Allergies. Cureus. 2019;11(4):e4577. Epub 20190430. doi: 10.7759/cureus.4577. PubMed PMID: 31281760; PubMed Central PMCID: PMC6605692.
22. Farzam K, Nessel TA, Quick J. Erythromycin. StatPearls. Treasure Island (FL): StatPearls Publishing
Copyright © 2022, StatPearls Publishing LLC.; 2022.
23. Jasper AS, Musuuza JS, Tischendorf JS, Stevens VW, Gamage SD, Osman F, et al. Are Fluoroquinolones or Macrolides Better for Treating Legionella Pneumonia? A Systematic Review

and Meta-analysis. Clin Infect Dis. 2021;72(11):1979-89. doi: 10.1093/cid/ciaa441. PubMed PMID: 32296816; PubMed Central PMCID: PMC8315122.