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THE STORY OF *LEGIONELLA*: FROM SURVIVING INSIDE AMOEBAS TO CAUSING PNEUMONIA

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YOUNG REVIEWERS:

ABED
AGE: 10

ABDULLAH
AGE: 12

SALLY
AGE: 15

Legionella pneumophila is type of bacteria present in natural water sources, such as rivers, ponds, and in man-made water reservoirs, such as water fountains. Amoebas are single-celled organisms that also live in water sources and generally feed on bacteria. However, *Legionella* are not digested by amoebas and have even evolved to multiply inside the amoebas that try to digest them! This resistance to being eaten by amoebas has enabled the *Legionella* bacteria to survive in water sources and to be transmitted to humans through contaminated water droplets. Their ability to survive inside amoebas also allows *Legionella* bacteria to survive and multiply within cells in the human lungs, leading to pneumonia. Therefore, the long relationship between *Legionella* and amoebas helped *Legionella* to evolved and to infect human macrophages causing disease.

WHAT IS LEGIONNAIRES' DISEASE?

In the summer of 1976, an unknown disease began spreading among hotel guests in the American city of Philadelphia [1–3]. Symptoms included feeling short of breath, coughing, chills, headache, chest pain, and sometimes diarrhea. One hundred eighty people were infected and 29 of them died as a result of this mysterious disease, including several members of the American Legion, an organization for US war veterans.

The US Centers for Disease Control and Prevention (CDC) immediately stepped in to determine the cause of this mysterious disease. Researchers took samples of lung tissue from the deceased victims and tried to figure out whether the infectious agent was an inhaled bacteria, virus, or fungi. After 5 months of experiments, the scientists identified the cause of the mysterious epidemic, and to their surprise it was a bacteria normally present in nature that was not previously known to infect humans. The scientists called it *Legionella pneumophila*—"*Legionella*" for the American Legionnaires who were infected, and "*pneumophila*" to indicate its infection of the lungs. The disease caused by these bacteria was termed Legionnaires' disease.

But how were the patients infected with these bacteria? Researchers knew that, in nature, *Legionella* is found in watery environments like rives and swamps. So, one theory was that patients inhaled water mist contaminated with pneumonia-causing *Legionella*, either inside the hotel or in the surrounding area. After collecting samples from water tanks, humidifiers, and water-cooling towers in the hotel's air-conditioning system, it was found that the cooling towers of the hotel's central air-conditioning system were contaminated with this *Legionella pneumophilia*.

AMOEBA

An irregularly shaped, single-celled organism often living in soil, and warm fresh water.

FOOD VACUOLE

Food vacuole is a membrane-enclosed sac, formed by the outer membrane of the cell after phagocytosis. The vacuole contains digestive enzymes, which break down the food and then it is released into the cytoplasm for utilization. Food vacuole is present in unicellular protozoans such as amoeba.

FROM AMOEBAS TO HUMANS

But why did humans suddenly become a new target for *Legionella*? To answer this question, we must go back millions of years, when *Legionella* existed in its natural aquatic environment with many other microorganisms, including a single-celled organism called an **amoeba**. Amoebas primarily eat bacteria present in their aquatic environments. Amoebas eat bacteria by surrounding them and bringing them inside the amoeba, creating a membrane-enclosed compartment called a **food vacuole**, the bacterium then is broken down by digestive enzymes inside the vacuole (Figure 1A). However, over millions of years, *Legionella* bacteria evolved in ways that helped them to avoid being eaten by amoebas—and even to control and manipulate many cellular processes within the amoeba! For example, *Legionella* bacteria evolved the ability to survive and reproduce inside of amoebas (Figure 1B), and inside other amoeba-like organisms

MACROPHAGES

Cells of the immune system, whose main function is to swallow and digest foreign bodies such as bacteria and viruses.

including cells called **macrophages**, which are part of the body's **immune system** and can be found in human lungs (Figure 1C) [4].

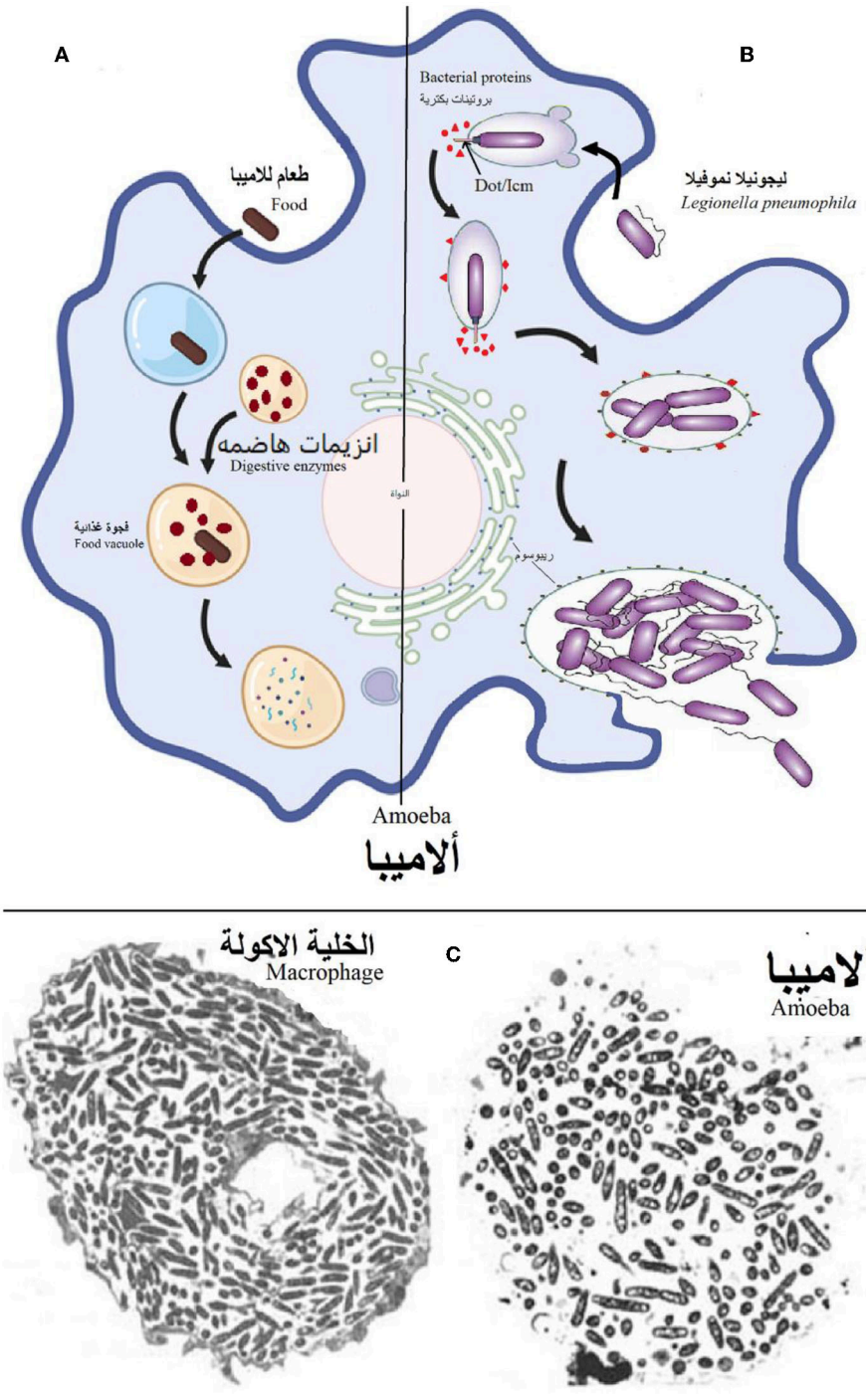

Figure 1

(A) When an amoeba ingests food (including most bacteria) from the surrounding environment, the amoeba breaks down the food using digestive enzymes, inside a food vacuole. **(B)** However, when an amoeba ingests *Legionella pneumophila*, the bacteria rapidly inject a large group of bacterial proteins into the amoeba, *via* the Dot/Icm "injector." These proteins target cellular processes within the amoeba and prevent the *Legionella* from being digested—instead, creating an environment that is suitable for their multiplication. After multiplying in large numbers, the *Legionella* bacteria emerge from the amoeba, ready to infect new cells and repeat the process. **(C)** Electron microscope images showing the multiplication of *Legionella* within a macrophage (left) and amoeba (right).

IMMUNE SYSTEM

Immune system is a complex network of cells, tissues, and organs. Together they help the body fight off the germs (bacteria or viruses) that invade your body.

The development of artificial water systems, such as air conditioning systems, cooling towers, fountains, showers, and other devices that release spray, have helped *Legionella* to infect humans. When a person inhales water spray contaminated with *Legionella pneumophila*, the bacteria reach the lungs, and as a defensive mechanism, the immune

cells present in the lungs—specifically macrophages—attack and devour the *Legionella pneumophila* to eliminate it. Since *Legionella pneumophila* has evolved to survive in amoeba-like cells, it begins to control the macrophages and multiply within them. This leads to pneumonia.

Elderly people and people with weakened immune systems are at the highest risk of developing Legionnaires' disease [5]. Fortunately, infected people can recover by taking **antibiotics**—medicines that kill bacteria. *Legionella* bacteria cannot spread between humans, so infected people cannot transmit the disease to healthy people.

ANTIBIOTICS

Antibiotics are medicines that fight bacterial infections in people and animals. They work by killing the bacteria or by making it hard for the bacteria to grow and multiply.

HOW DOES *LEGIONELLA* TAKE CONTROL OF CELLS?

How does *Legionella pneumophila* manage to survive and multiply inside amoebas and macrophages? Scientists have discovered that one of the most important factors is an integrated structure in the plasma membrane of *Legionella*, called **Dot/Icm** (Figure 2). This structure looks like a needle, and it is used to inject more than 350 bacterial proteins from the *Legionella* into the amoeba or macrophage. These bacterial proteins interfere with the normal cellular activities of amoebas or macrophages, preventing those cells from digesting *Legionella* and creating the conditions that allow the *Legionella* to survive and multiply. To understand how those bacterial protein can control cellular activities of amoebas or macrophages, studying the DNA of *Legionella pneumophila* was essential. Therefore,

DOT/ICM

Defect in organelle trafficking/intracellular multiplication.

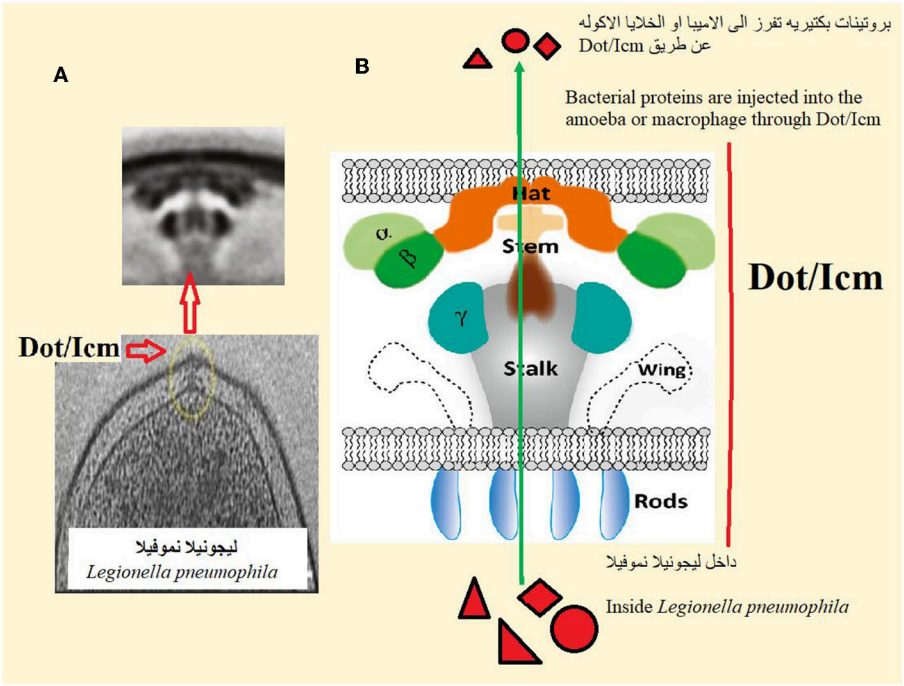

Figure 2

(A) Dot/Icm is a structure on the surface *of Legionella pneumophila* that "injects" bacterial proteins directly into amoeba or macrophage. The upper image is an enlargement of the circled area in the lower image. **(B)** Dot/Icm consists of several parts that work together to allow it to inject bacterial proteins.

scientists discovered that 5% of *Legionella pneumophila* DNA codes for bacterial proteins are similar in structure and function to proteins found in the amoebas or macrophages they infect. These similar proteins probably play a role in interfering with the cellular functions of infected cells, allowing *Legionella* to survive and replicate.

Where did these important genes—so similar to those of amoebas and macrophages—come from? Over millions of years, *Legionella* likely acquired those genes after it was taken up by amoebas, or from the watery environment in which both organisms were living. *Legionella* is known to be able to uptake pieces of DNA that are present in the watery environments, regardless of the type of organism they come from!

CONCLUSION

Legionella pneumophilia is a fascinating organism that exists naturally in watery environments. But, with the development of artificial water systems over the past few decades, the chances of human exposure to water spray contaminated with *Legionella* has increased. Since the macrophages in human lungs are similar to the amoebas that *Legionella* has evolved to infect in nature, some of the proteins produced by these bacteria can control the cellular activities of macrophages, allowing *Legionella* to avoid digestion and multiply within human macrophages, leading to pneumonia. It can be said that the long relationship between amoebas and *Legionella* helped *Legionella* to both survive in nature and to infect (and cause disease in) humans.

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YOUNG REVIEWERS

ABED, AGE: 10
Abed wants to become a doctor when he grows up. He is from the Kingdom of Saudi Arabia, Mecca in particular. He loves reading and enjoys discussing what he reads with friends. He enjoys reading stories, novels, and science, especially biology. Abed has many hobbies such as swimming and football. He is also talented in drawing.

ABDULLAH, AGE: 12
Abdullah wants to become a doctor when he grows up. He is from the Kingdom of Saudi Arabia, Mecca in particular. He loves reading and enjoys discussing what he reads with friends. He enjoys reading stories, novels, and science, especially biology and physics. Abdullah has many hobbies such as swimming and football. He is also talented in drawing.

SALLY, AGE: 15
Sally is 15 years old, and she is in year 10 in the British school in Riyadh. She is interested in studying medicine and wishes to become a surgeon in the future. In her free time, Sally likes to play the piano and study chemistry and astronomy. She also likes swimming.

AUTHORS

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Tasneem holds a Ph.D. in immunology and microbiology from the University of Louisville in Kentucky, and a master's degree in medical laboratory analysis from the University of Jordan. She worked for 4 years as a full-time lecturer at the Hashemite University in Jordan, then moved to the United States and is currently working as a researcher at the University of Louisville, in the field of microbiology. Her research interest is in the field of *Legionella pneumophila* and its ability to acquire food to survive and reproduce within the amoeba. *tfalqu01@louisville.edu

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Professor Youssef Abukwik is of Palestinian origin, born and raised in Old Jerusalem. He was the first member of his extended family to receive a high school diploma. He received his bachelor's degree in medical technology from Yarmouk University in Jordan. A Fulbright scholarship for postgraduate studies enabled him to study in the United States, and thanks to this scholarship he obtained both his master's and doctoral degrees in molecular microbiology from the State University of New York at Buffalo, followed by post-doctoral training at the University of Michigan. He is currently a Distinguished Professor at the University of Louisville, in Kentucky. Throughout his career, his research has focused on the molecular interaction of pathogenic bacteria with human phagocytes or amoebas. Prof. Youssef supervised twenty Ph.D. theses and published 140 research papers in famous scientific journals. Also, he is the founder and the Field Chief Editor of Frontiers in Cellular and Infection Microbiology.