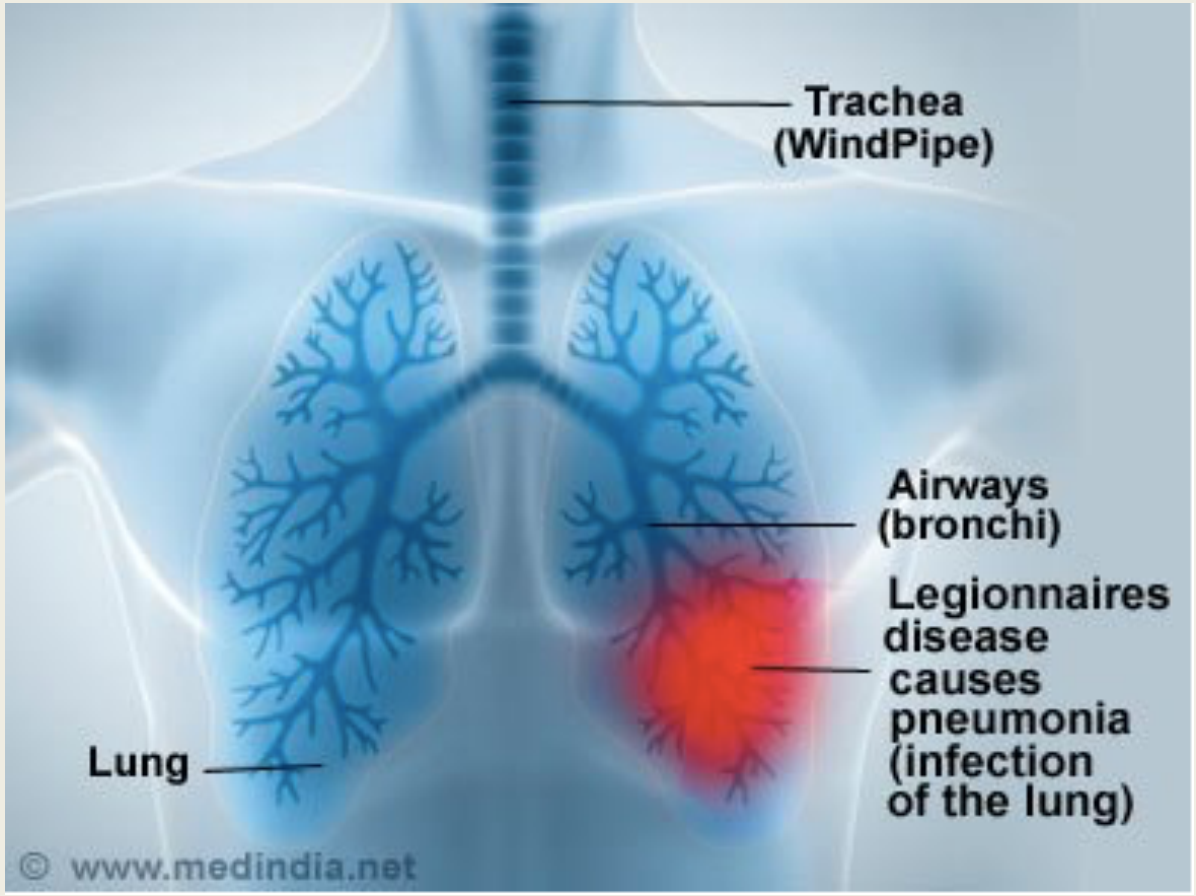

Image retrieved from Siegel 2018

A CRUISE HOLIDAY

Case 3 The Immune Response

Summary by Sabrina Budhwani

The Case: A Cruise Holiday

To celebrate Tom's retirement his wife and two adult children accompany him on a long anticipated cruise. Tom's asthma flares up a few days before the cruise but with a corticosteroid nebulizer in tow he feels well enough to join the cruise. Even more than the rest of his family, Tom enjoys the various hot tubs aboard the massive ship those first few days, relishing the relaxation after a busy final year at work. On the fifth day of the cruise, Tom wakes up in a sweat with a cough that continues throughout the day. As the day wears on he feels worse with a headache, muscle aches and nausea accompanying the cough. His wife arranges for the cruise doctor to visit him in his cabin. The doctor examines Tom, notes his high temperature, relatively nonproductive cough and recent history of asthma and corticosteroid therapy. She takes a full history including taking note of his activities during the first days of the cruise and diagnoses Tom with a pneumonia. She explains that her presumptive diagnosis is that of Legionnaires disease and leaves Tom's wife with a sterile sample container to collect whatever fluid Tom might cough up for delivery to her. She explains that she can do a microscopic examination on the respiratory fluid which will help in the diagnosis. In the meantime she starts Tom on erythromycin and lets the family know that she will check in on Tom regularly over the next few days to monitor his progress. More people are diagnosed with a similar pneumonia over the next two days, mostly in people who came aboard with a slightly compromised immune system, like in Tom's case. The cruise ship alerts the hospital at their next port of call in case any of the patients worsen enough to require hospitalization. When they arrive at port blood samples are collected from all of the patients and delivered to the hospital laboratory for serology. The ship also takes extra time in port to allow for a full scale sterilization regime to be performed on all of the hot tubs. At this stage Tom is feeling well enough to continue on the cruise, although at a slower pace than when he first boarded.

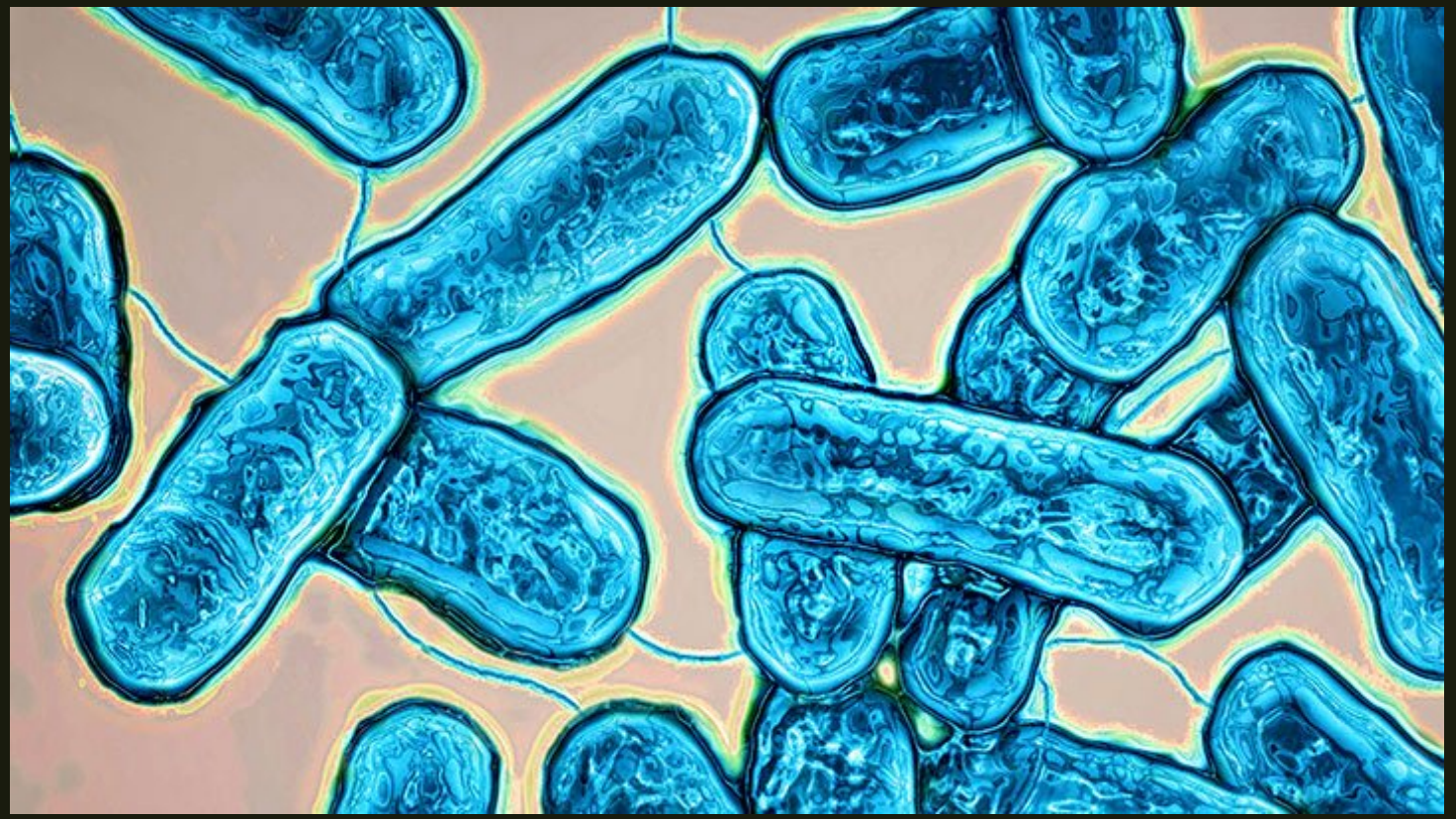

Figure 1: retrieved from Phelps 2016

Legionnaires' Disease

- Severe pneumonia
- Pneumonia is lung inflammation caused by bacterial or viral infections in which the air sacs fill with pus and may become solid
- Legionnaire's disease is caused by the gram-negative bacterium *Legionella*, usually *Legionella pneumophila*
- Legionnaire's disease targets those with weakened/compromised immune systems

WHAT ELEMENTS OF THE INNATE AND ADAPTIVE (HUMORAL AND CELLULAR) IMMUNE RESPONSE ARE INVOLVED IN THIS INFECTION?

Two Types of Immune Responses

Innate Immune Response

- Nonspecific response
- Fast activation via antigenic detection
- Pathogen Associated Molecular Patterns (PAMPs) are recognized by Pathogen Recognition Receptors (PRRs)

Adaptive Immune Response

- Specific response
- Delayed activation via antigenic detection
- Antigen is processed, displayed, recognized and attacked
- Memory is attained to the antigen

Innate Immune Response

- First line of defence against pathogens
- Immediate and non specific immune response
- Pathogen comes into contact with airway epithelium (physical barrier from environment)
- Epithelial cells have PRRs (pathogen recognition receptors) like TLR's (toll like receptors) or NLRs (nucleotide binding oligomerization domain like receptors) which detect and bind to PAMPs (pathogen associated molecular patterns) on the pathogen
- After this detection and binding, the body produces antimicrobial compounds and cytokines to fight the pathogen
- **TLR2** is important in recognition of bacterial peptidoglycans and lipoproteins

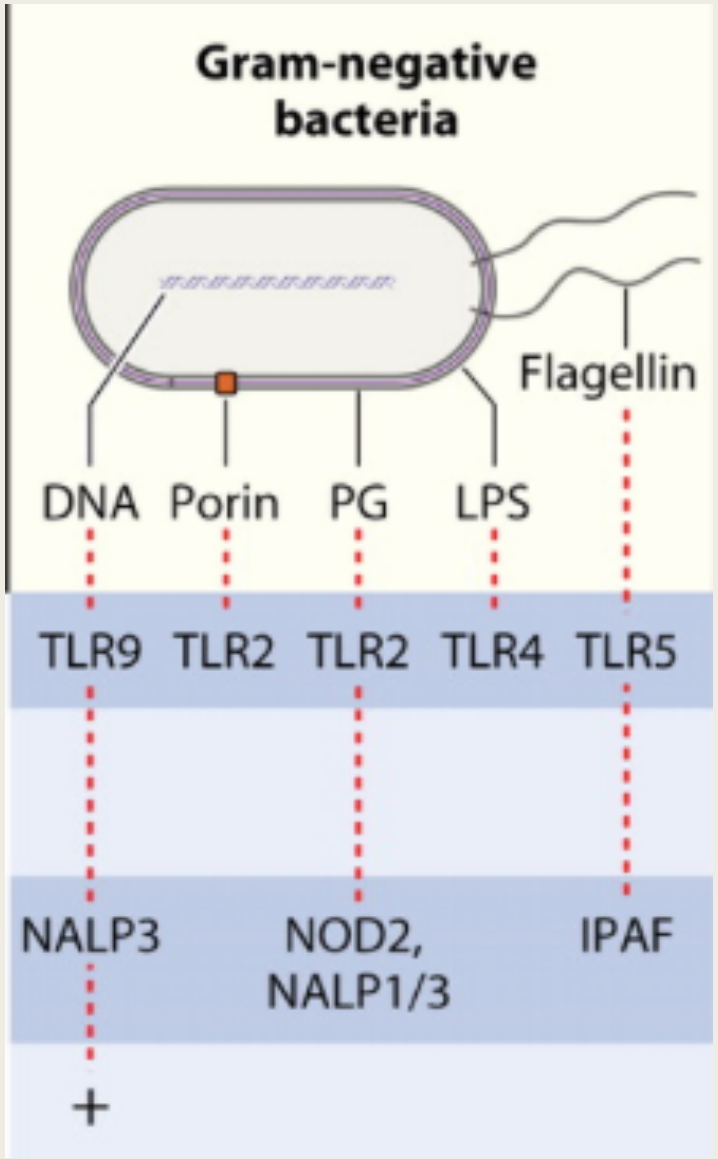

Figure 2: Shows TLRs and NLRs response in recognition of PAMPs such as flagellin with **IPAF** Retrieved from Mogensen 2009

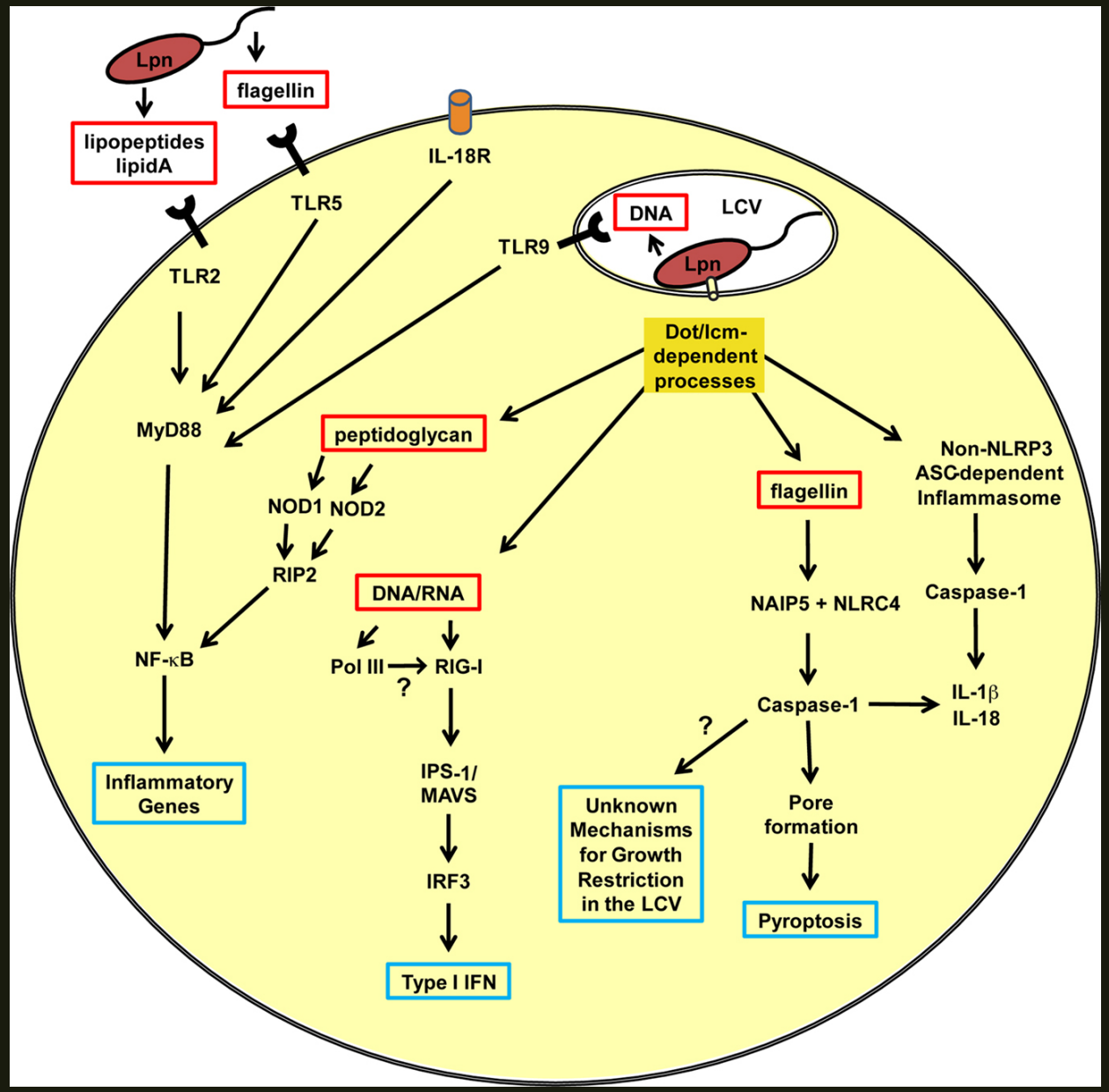

Figure 3 retrieved from Massis 2011

- Figure 3 shows the *Legionella pneumophilla* PAMPs in red boxes, such as flagellin, DNA/RNA, peptidoglycan and lipopeptides which are recognized by the PRRs in the epithelial cells. Retrieved from Massis 2011.

Innate Immune Response in Legionella

- **NLRs :** NAIP4 and IPAF recognize the flagellin of *L. pneumophila* (PAMP)
- These PRR's restrict bacterial replication in macrophages and epithelial cells
- **Cytokines** are then released, epithelial cells rely on **nuclear factor-kB** (refer to figure 3) to express different cytokines which attract neutrophils to the site of infection
- **Neutrophil recruitment:** CXCL5, GM-CSP
- **TLR's** in the epithelial cells signal to activate NF-kB – it induces pro-inflammatory cytokines and **mucin** which trap particles in the airway
- Cilia push out the mucus from the epithelial cells out of the lungs so the inhaled particles can be trapped, **antimicrobial compounds in mucus are IgA antibodies, collectins and defensins**
- **Resident macrophages: initiate inflammatory response and present antigens to B cells and T cells in the adaptive immune response**

Adaptive Immune Response in *Legionella*

- **Resident Macrophages:** activate T and C cells by recognition of MHCs on antigen presenting cells which produce antigen specific antibodies for future infections
- **Cytokine production**: interferon gamma-y (**INF-y**), tumor necrosis-a (**TNF-a**) and interleukin (**IL-6 and IL-1**) facilitate inflammatory response against the bacteria in this case *Legionella*
- **T cells** receive these cytokine signals from the macrophages and differentiate into Th1 and Th2 cells
- **Th1** = produce interferon-y which increases production of reactive oxygen and nitrogen species and controls the growth of intracellular pathogens. They recruit other Th1 cells by expressing **CXCL9, CXCL10 and CXCL11** which can help form complex immune responses.
- Reactive oxygen species (hydroxyl and alkoxyl) degrade proteins and regulate apoptosis.
- **Th2** = controlled by **GATA3 and STAT6** , they express **IL-4** (B cell proliferation and upregulation of MHCII) ,**IL-5** (regulating eosinophils) and **IL-13**

- B cells are activated by APCs and form plasma cells which make antibodies against the bacteria – *Legionella* to fight the infection and create memory cells (seen in figure 4)
- Antibodies act as markers so macrophages can identify them efficiently
- Antibodies in mothers breastmilk can also supplement infant immunity

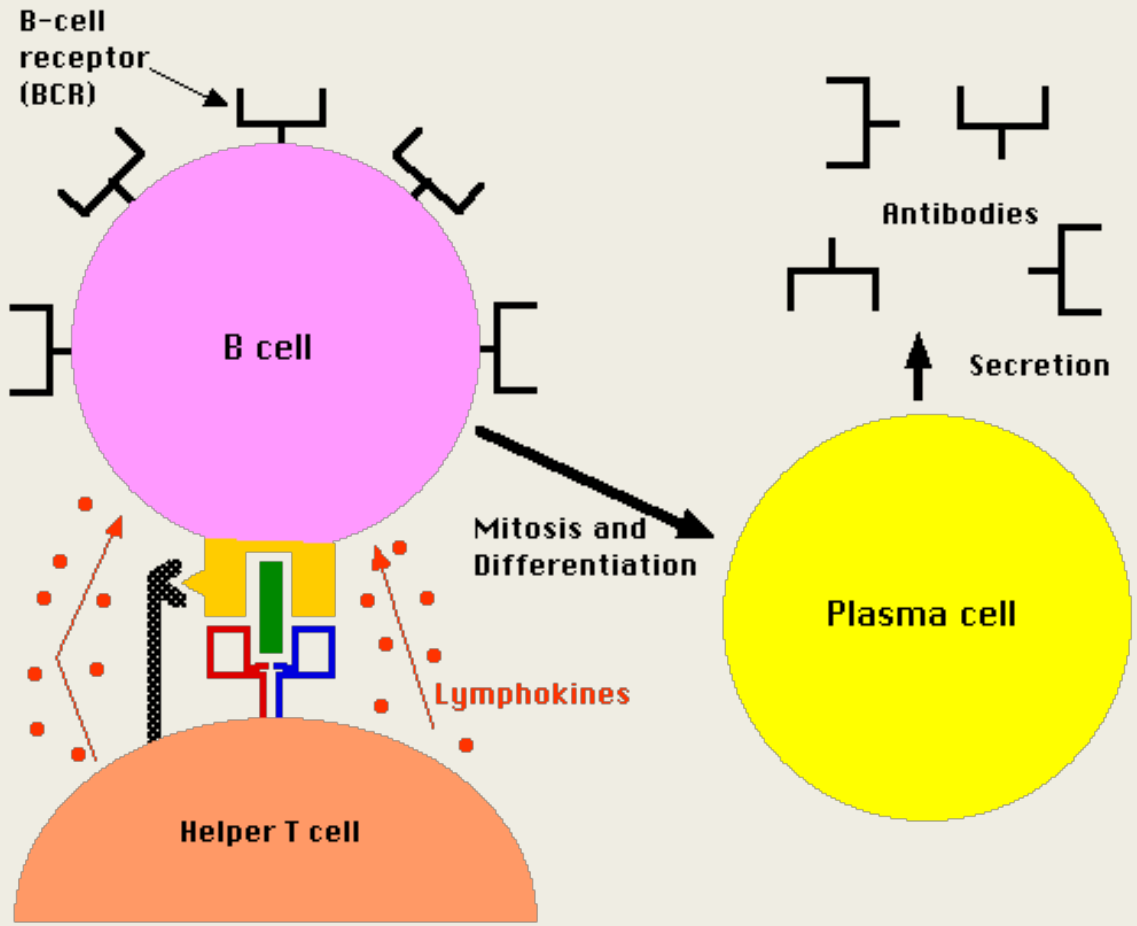

Figure 4: Retrieved from Biology Pages 2005

HOST DAMAGE: WHAT DAMAGE ENSUES TO THE HOST FROM THE IMMUNE RESPONSE?

- The **cytokines** produced from the innate response aid in protection to the host, but high elevation of cytokine concentrations can be fatal to the host causing sepsis and toxic – like death
- **Septic shock**: TNFalpha and IL-1 are central mediators and produced in high concentrations, because of LPS stimulating host immune cells
- Increased pro-inflammatory cytokines can lead to irregular coagulation, hypotension leading to respiratory distress syndrome

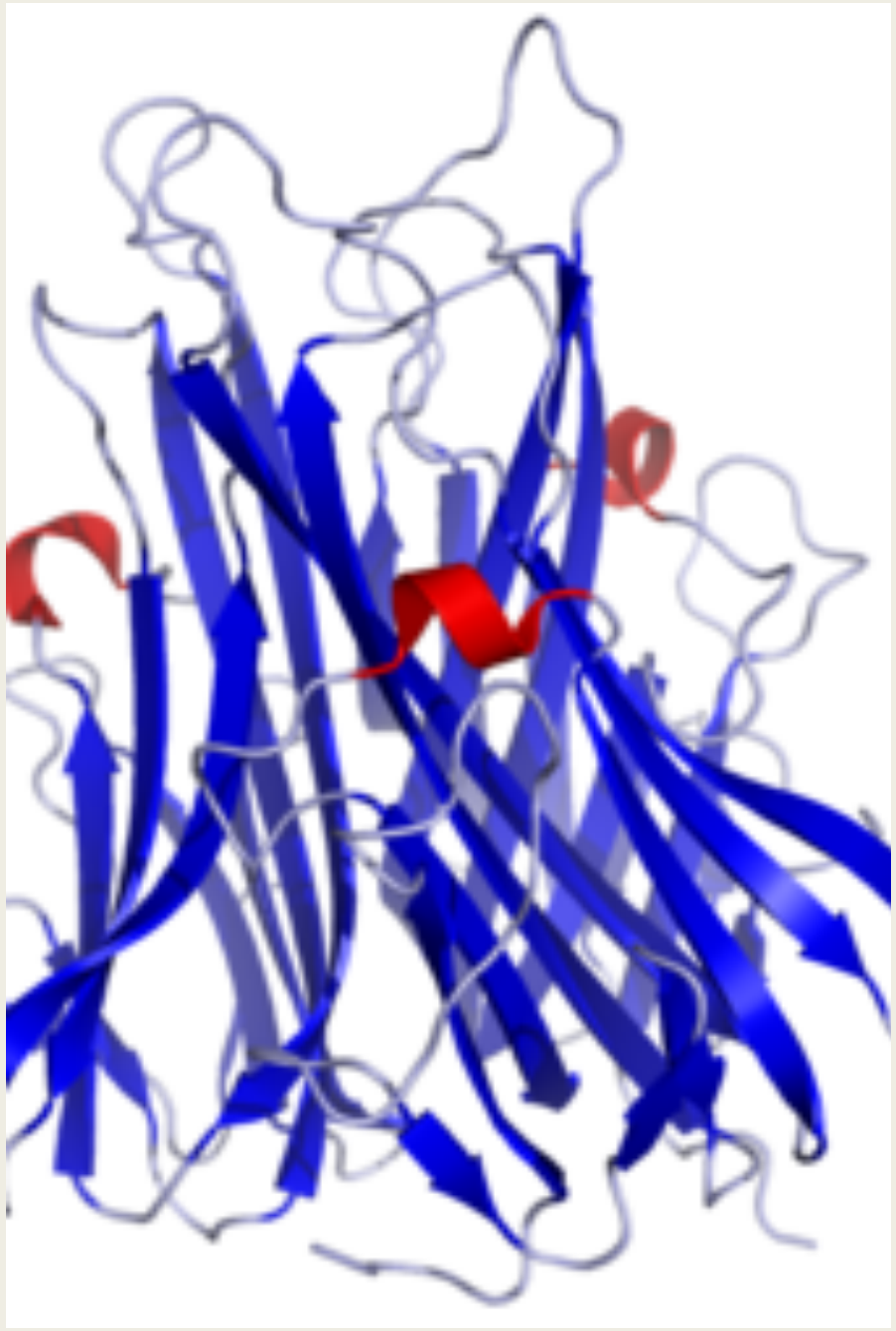

Figure 5 showing structure of TNF-alpha retrieved by Wiki 2017

- During the adaptive immune response dendritic cells activate **CD4+ and CD8+ T cells** which damage localized tissue
- Infection with *Legionella pneumophilla* often results in cell death of **macrophages** to inhibit the spread of this bacteria, **macrophage pryptosis** (form of apoptosis which is caspase-1 independent) starts with NLRC4 inflammasome activation. This complex initiates fusion of infected **macrophages** with the **phagolysosomes** leading to cell death
- In an infection, inflammatory cells are increased in number which can itself lead to **damage to the epithelial layer and increase its permeability causing fluid build up in the interstitial and alveolar spaces due to pulmonary edema**
- This fluid buildup results in **dyspnea due to restriction of proper gas exchange**
- ***Legionella pneumophilla*** can cause **massive pulmonary consolidation and necrosis,** this condition can be fatal if not treated

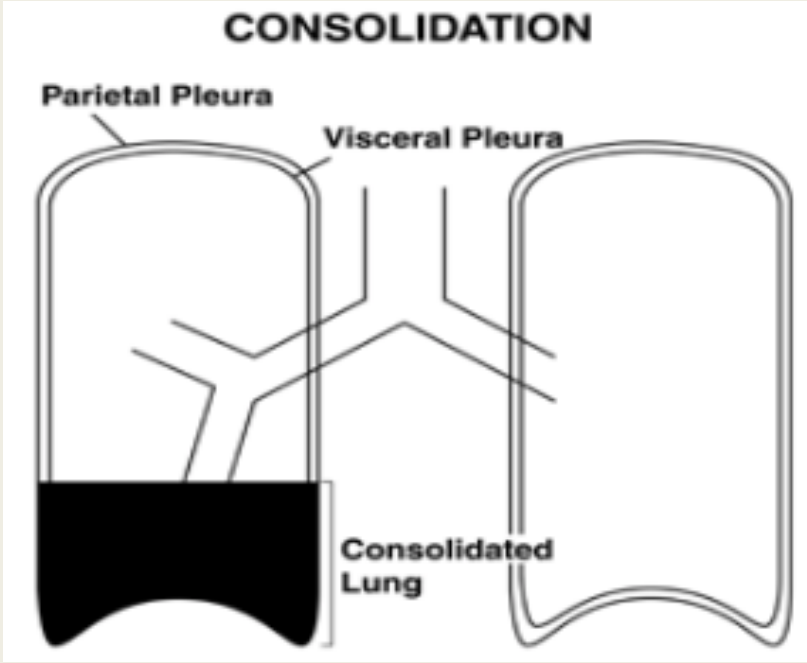

Figure 6 shows consolidation, when lung parenchyma fills with fluid or tissue, mainly seen in pneumonia. Retrieved from UCSD school of medicine (2015)

BACTERIAL EVASION: HOW DOES THE BACTERIA ATTEMPT TO EVADE THESE HOST RESPONSE ELEMENTS?

1. CELL ENVELOPE
2. SURVIVAL INSIDE THE HOST CELL
3. KILLING ALVEOLAR MACROPHAGES

1. Properties of the *Legionella* cell envelope (A)

- Cell surface of *Legionella* contains common structures found on many bacterial surfaces: **lipopolysaccharide (LPS), outer-membrane vesicles (OMVs), flagella and type IV pilli**
- **OMVs :** important in early stages of infection, facilitates binding, transfer and fusion of bacteria to the host surfaces – allows for engulfment by the host **since *L.pneumophilla* is an intracellular pathogen, it must find a way to avoid being degraded after being phagocytosed. *L. pneumophilla*** does this by altering the pH of its phagocytic vacuole and the OMV **prevents fusion of the phagosome with the lysosome**
- **Type IV pili :** involved in **attachment and entry** of bacteria into host cell, **promotes biofilm development and adhesion** (increases *L. pneumophilla* survival)

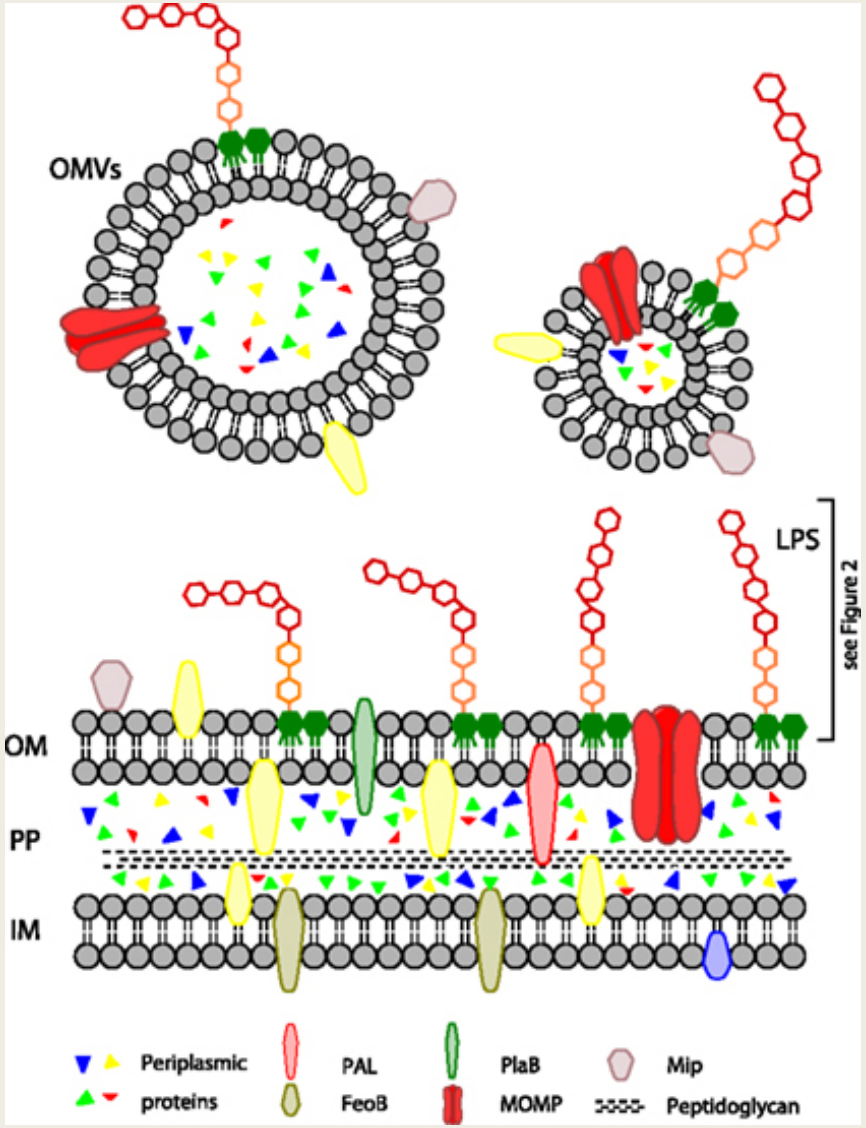

Figure 7 shows L. pneumophilla's cell envelope with LPS, OMVs and Mip protein. Retrieved from Shevchuk 2011.

1. Properties of the *Legionella* cell envelope (B)

- Bacterial surfaces contain antigenic targets which can initiate an immune response by the host due to TLR recognition, such as complex surface proteins and carbohydrates
- Bacteria disguise their surface antigenic targets by **expression of a carbohydrate capsule**, this is mainly done by extracellular bacterial pathogens since they systematically circulate in the body and keep their **bacterial surface hidden from the host immune system** but have filamentous adhesins like fimbriae and pili which protrude out of the capsular surface to ensure adhesion to the host.
- **LPS :** main surface exposed structure of gram negative bacteria. **Contains Lipid A – plays a key role in activating TLRs**, the outer component of LPS has varying carbohydrates which differs from each strain – different serotypes (O antigen). **This is how same species can re infect the same host**
- **Secretion systems: export virulence factors across the bacterial cell into host cells or the environment. T3SS and T4SS** insert molecules into the host such as toxins, immune modulators, paralyze phagocytosis, enhance intracellular parasitism and re-program vesicular transport.

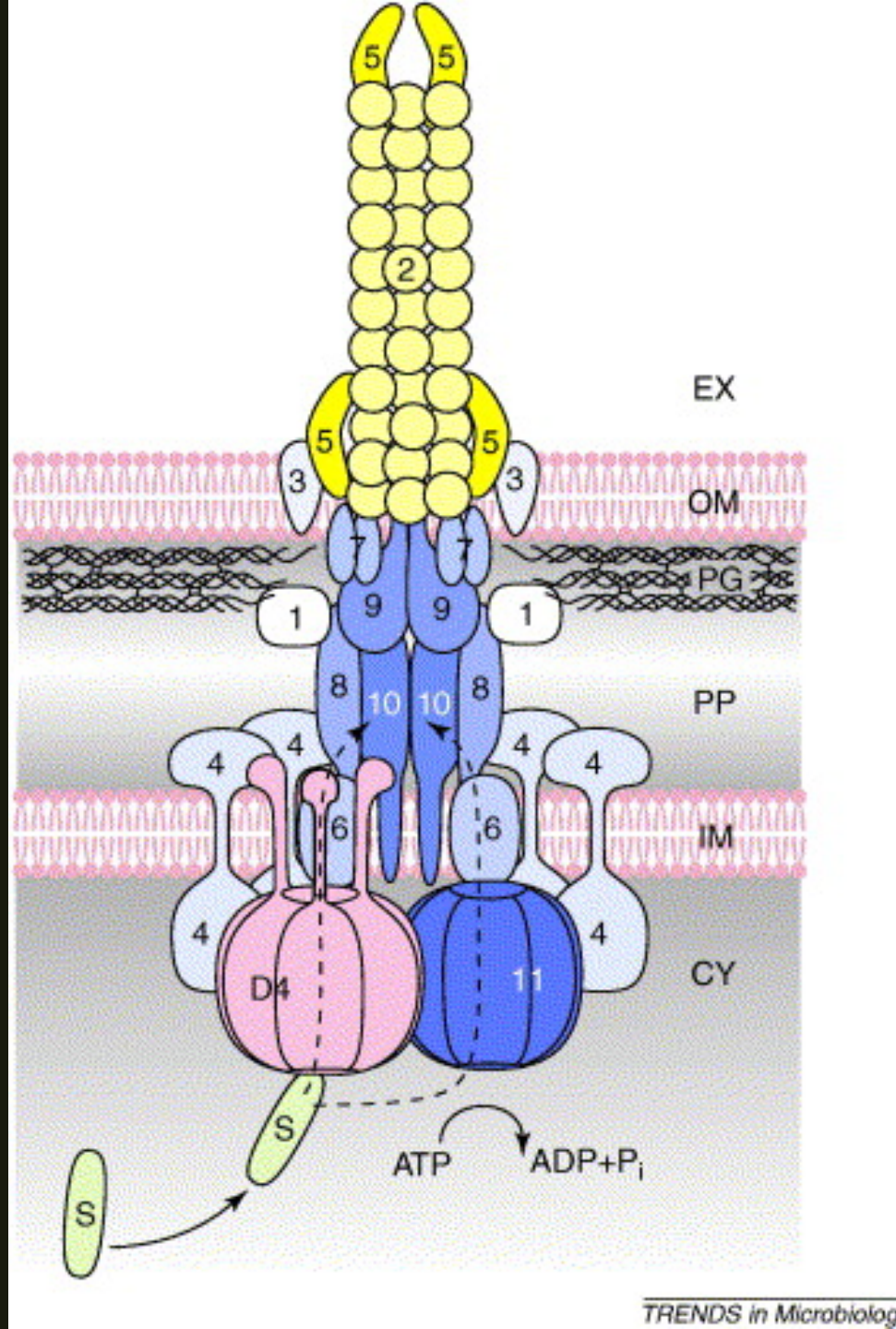

Figure 8: Shows the T4SS. Retrieved from Schroder 2005

2. Survival inside the host cell

- ***Legionella*** survive and replicate within a host cell and block killing of the infected host cells. When *L. pneumophilla* infects a host cell and needs to replicate, it must **prevent activation of this infected cell first.** This is done by **Mip protein** which imitates the function and structure of the eukaryotic protein family FK506.
- ***Legionella*** also uses **Dot/Icm type IV secretion system:** used to invade the host and create signal molecules which facilitate the growth of *Legionella.* **The type IV secretion system inhibits host cell apoptosis and promotes intracellular replication of bacteria**
- ***Legionella containing vacuole (LCV):*** *created when* bacteria release bacterial effector proteins into the cytosol once phagocytosis is initiated by the alveolar macrophage. **LCV prevents recruitment of immune cells to the infected cell, creating a safe replication site for the bacteria. *L. pneumophilla* avoids phagolysosome fusion and replicate in alveolar macrophages, LCV don't get acidified so *Legionella* can safely divide within these macrophages.**

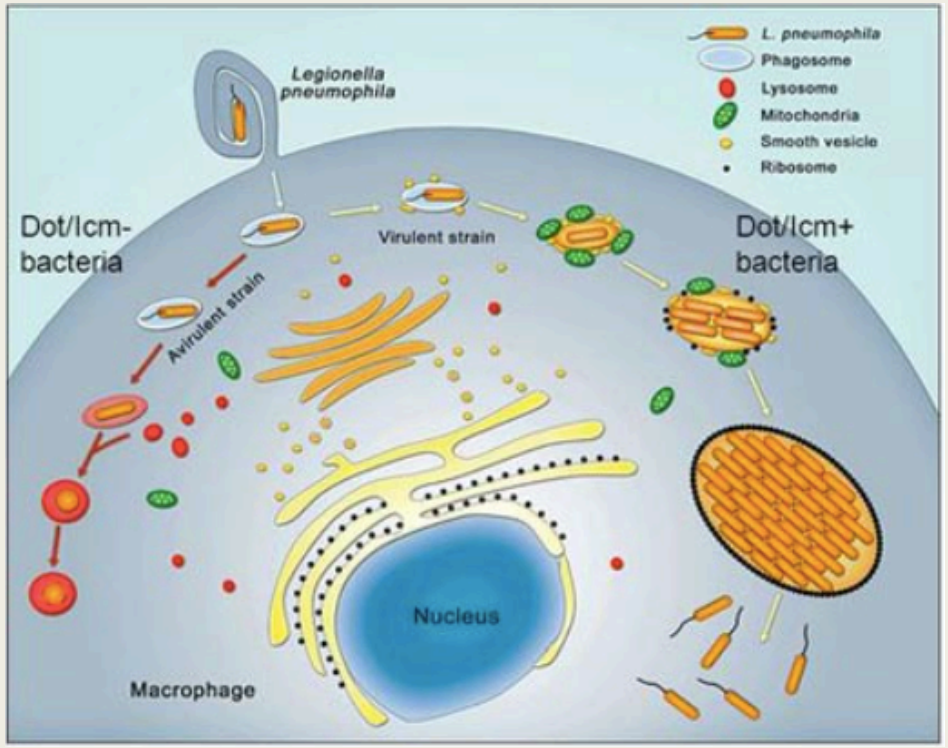

Figure 9 shows Dot/Icm T4SS in *L. pneumophilla* Retrieved from Aryal 2018.

3. Killing Alveolar Macrophages

- Since **Legionella** replicate within alveolar macrophages, this leads to lysis of these host cells (death) causing high numbers of replicated bacteria to be released
- This leads to an advantage for *Legionella* since they now have increased numbers of replicates and the host cell alveolar macrophages in the lungs are decreased causing a decrease in the host immune response to *Legionella* entering the airways

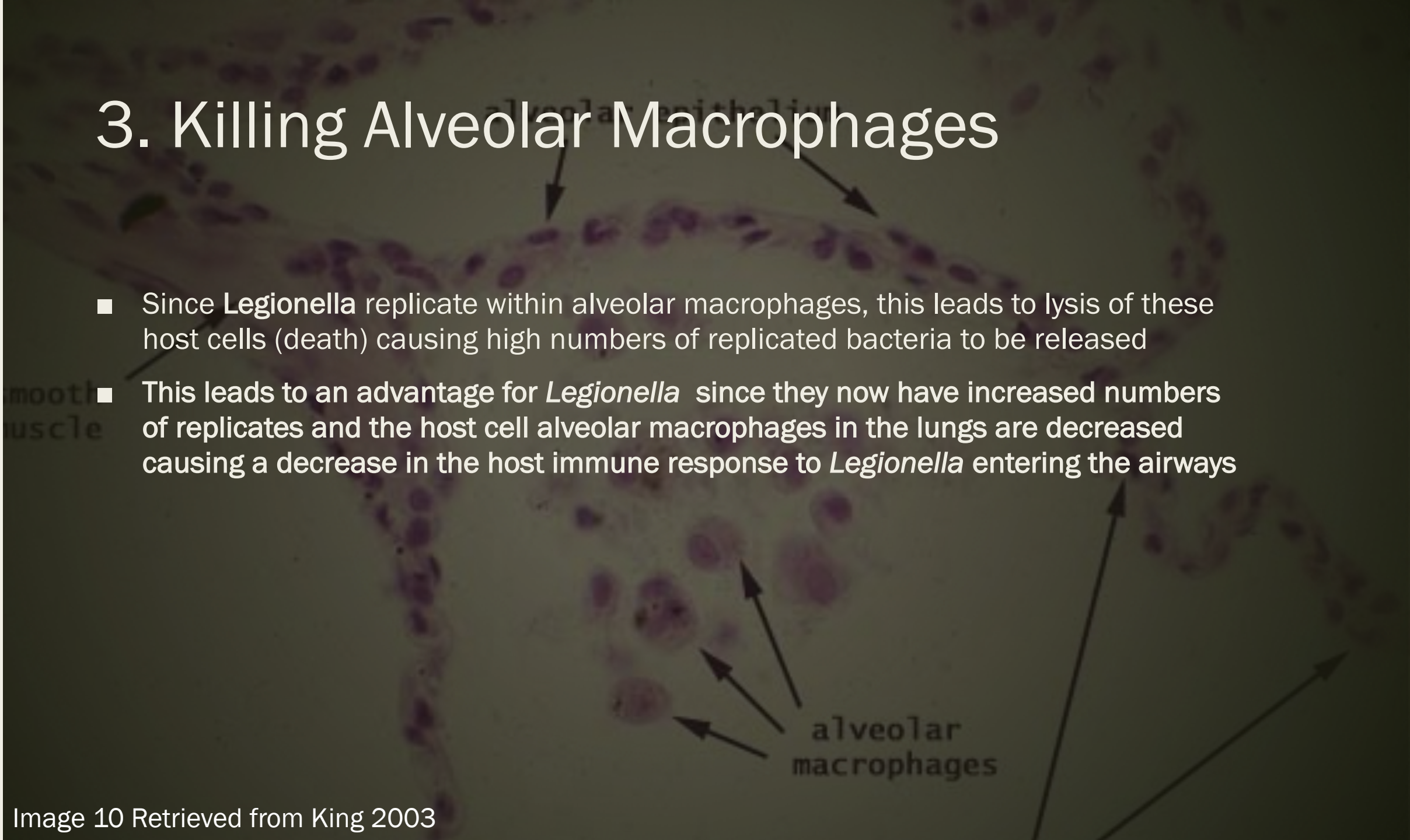

Image 10 Retrieved from King 2003

OUTCOME: IS THE BACTERIA COMPLETELY REMOVED? DOES THE PATIENT RECOVER FULLY AND IS THERE IMMUNITY TO FUTURE INFECTIONS FOR THIS BACTERIA?

- When an individual is infected with *L. pneumophilla* the adaptive immune system develops a strong B cell response specific to the pathogen. This response occurs rapidly following a secondary infection. CD4+ Th2 cells produce interleukin 4-6 and activate B lymphocytes, leading to differentiation to **plasma cells producing antibodies towards the specific pathogen, long lived plasma B cells/memory cells are also made for immunity towards the pathogen.** While keeping in mind that these bacteria can change their LPS carbohydrates- changing serotypes which then cant be recognized by the memory cells.
- Legionnaires disease can be deadly but if treatment is received in a timely manner, **90% of infections can lead to a full recovery.**
- Interferon- gamma made by T cells also aid in the removal of the pathogen from the host body
- Treatment with antibiotics, the bacteria is completely removed from the body and full recovery is achieved but those with **scarring of lung tissue from the infection will not fully recover**

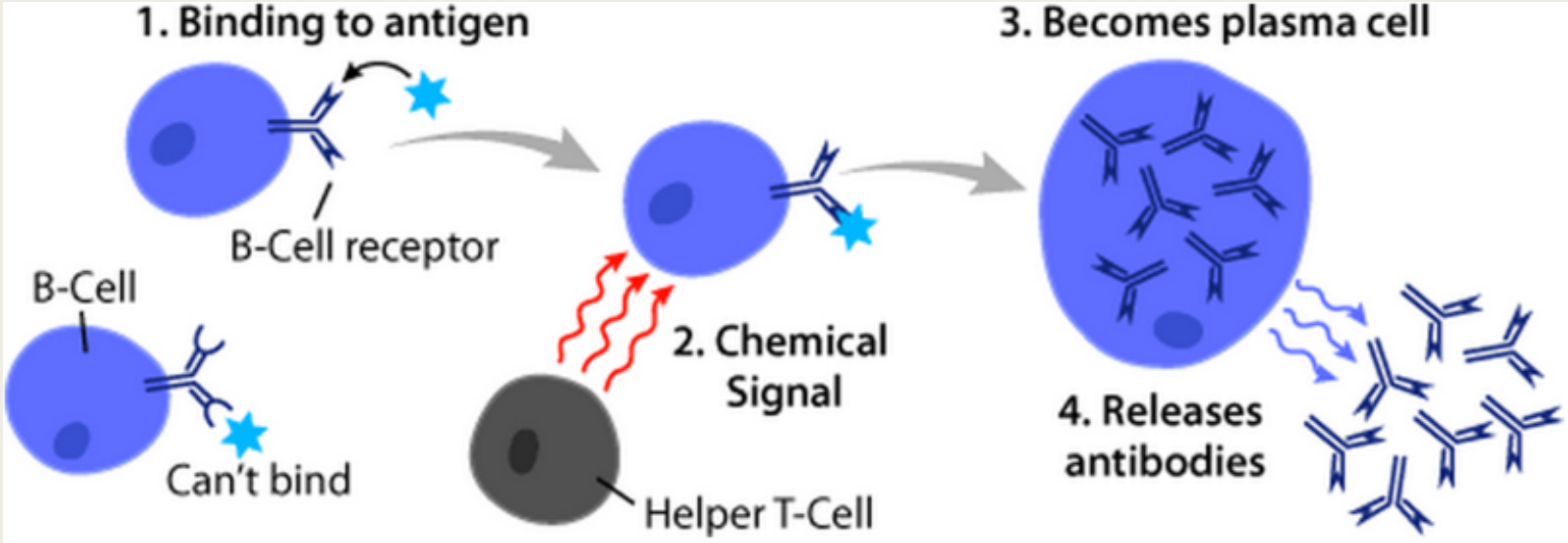

Figure 11 shows B cell function in producing antibodies. Retrieved from Wiki 2017.

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