Hilury Ha
PATH 417A

Case 3 Q4– The Immune Response

Introduction

Robert is a 53 year-old Indian immigrant that presents with a fever of 38.5^{o}C, chills, night sweats, and chronic productive cough over the past month. Auscultation results indicate crackles in the right lung and decreased breath sounds in the right lower lung field. His symptoms and auscultation results suggest bronchitis, tuberculosis (due to infection by *Mycobacterium tuberculosis),* and pneumonia.

Bronchitis can be caused by bacteria such as *Bordetella pertussis, Streptococcus pneumoniae and Mycoplasma pneumoniae. B. pertussis* infection is ruled out in this case because Robert does not exhibit symptoms such as rapid and numerous coughing fits with a "whoop" sound lasting 1 to 3 weeks, or vomiting and exhaustion (Finger & von Koenig, 1996), although microbiology laboratory testing would be necessary to confirm the presence of this pathogen. In addition *M. pneumonia* primarily affects people under the age of 40 and upon chest auscultation exhibit localized rhonchi (Waites & Talkington, 2004), which leads this pathogen to be less likely responsible for Robert's illness. It is highly likely that *S. pneumoniae* is responsible for Robert's symptoms, as infection elicits distinctive symptoms such as cough and sputum production, fever (due to cytokine release), chills, and crackling sounds during auscultation (Musher, 1992). It is also of interest to note that there have been previous cases of high tuberculosis incidence rates in Indian immigrants (Roe, 1959). Also, those infected by *M. tuberculosis* exhibit symptoms of pulmonary disease: chronic productive cough, low fever and night sweat, as seen in Robert.

I. Host Response:

Streptococcus pneumonia

Human hosts defend themselves against infection by *S. pneumoniae* by physical features such as the mucociliary barrier, and immune responses such as phagocytic and inflammatory responses, T-dependent immune responses, and type-specific anti-capsule antibody release (Patterson, 1996). Human hosts possess antibodies to the M protein of group A streptococci which is used for attachment to host cells. Possessing type-specific anti-capsular antibodies to T-independent antigens allow for opsonisation and ultimately recovery after infection (Patterson, 1996).

The structural features of *S. pneumoniae* cell wall that elicit immune response include pneumococcal surface protein A (PspA), and the phosphorylcholine (PC) determinant of cell wall techoic acid (Figure 1) (Snapper et al., 2001). Research has shown that CD4 T-cell receptor αβ T-cells are capable of eliciting both polysaccharide and protein specific Ig responses (Snapper et al., 2001). IgG anti-PC and IgG anti-PspA, as well as anti-pneumolysin responses are dependent on TCR-αβ (Snapper et al., 2001).Upon exposure to the pathogen, anti-PC antibody response is often observed earlier than anti-PspA response; however they are both dependent on CD4 TCR-αβ T cells (Snapper et al., 2001). This is because the anti-PC response may not require the generation of new MHC class II- peptide complexes, instead the immune response is augmented by non-cognate activation of TCR-nonspecific T cells (Snapper et al., 2001). On the other hand, the anti-PspA response is dependent on cognate MHC class II interactions between dendritic cells and T cells (Snapper et al., 2001). In both cases, T cell induction requires

signalling from the T cell receptor by MHC-peptide complexes on antigen presenting cells as well as co-stimulatory molecules such as CD28 which binds to B7-1 and/or B7-2 on antigen presenting cells (Snapper et al., 2001). Physical interaction between T cells expressing CD28 and B7 generally occurs in the parafolllicular T cell zones of secondary lymphoid organs, therefore localizing the immune response (Snapper et al., 2001).

Toll-like receptors (TLR's) are receptors that are vital in mounting host immune responses to pathogens due to their ability to recognize conserved molecular patterns on foreign materials. For example, upon recognition of surface proteins such as lipotechoic acid on the cell wall of *S. pneumoniae* by TLR 2, downstream genes such as nuclear factor NF-kB are activated. Additionally, TLR-4 mediates the innate immune response to pneumococcal cytotoxin pneumolysin (Koedel et al., 2004). Contact with pathogenic markers by antigen presenting cells can upregulate the T cell and B cell response by inducing cytokine and tumor necrosis factor release in the infected area by mast cells, neutrophils and endothelial cells (Snapper et al., 2001), leading to the inflammatory response. The acute inflammatory response caused by the pathogen is beneficial because it increases the likelihood of pathogen transmission despite the increased risk of clearance by the host (Kadioglu, Weiser, Paton & Andrew 2008). The inflammatory response also leads to increased C-reactive protein (CRP) in the serum (Johnston, 1981). Interactions between CRP and *S. pneumoniae* surface proteins initiate the classic complement pathway and opsonisation (Johnston, 1981).

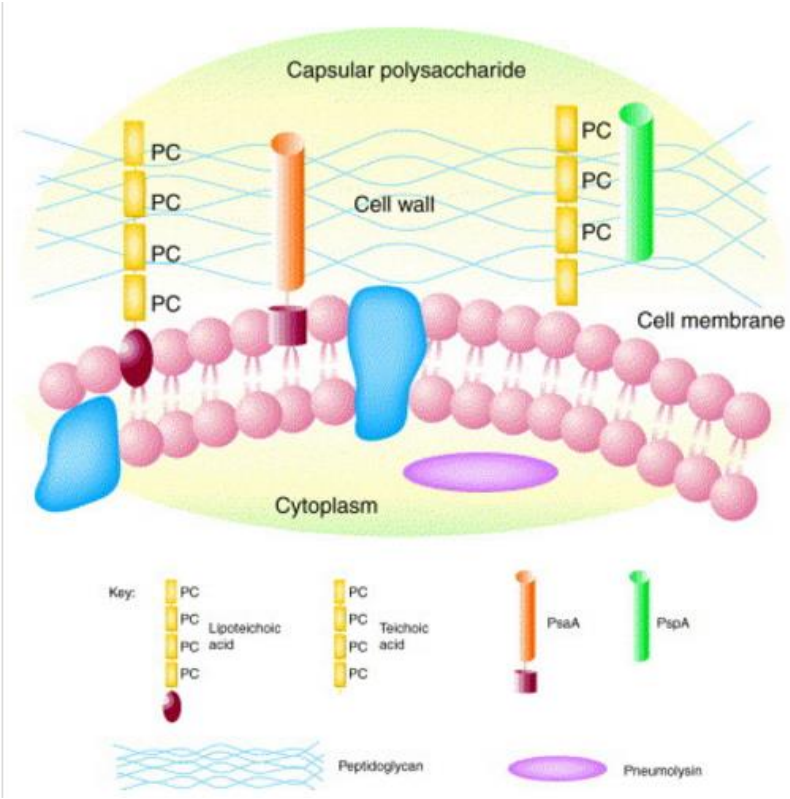

Figure 1. Structural features of *S. Pneumoniae* cell wall. Image reproduced from Snapper et. al., 2001.

Mycobacterium tuberculosis

Resistance to infection by *M. tuberculosis* is dependent on T cell activity (Figure 3) to lyse infected macrophages directly or activate them through soluble mediator release to destroy intracellular bacilli (McMurray 1996). Unlike in *S. pneumoniae* infection, antibodies do not play a protective role in the host immune response. Antibody mediated immune responses are not helpful in protecting the host from the pathogen because the bacteria reside intraceullarly and thus remain undetected; even if it is in the extracellular environment, its unique cell wall structure (high in lipids) renders it resistant to the complement mechanism (Todar, n.d.).

Acquired immunity after infection is mediated by T cells and develops within 4 to 6 weeks of infection (McMurray, 1996). Upon contact of macrophages with lipopolysaccharides on the bacteria cell surface, tumor necrosis factor (TNF) and interferon (IFN-ϒ) are released, which lead to reactive nitrogen intermediate production (Flynn et al., 1995). Studies show that tumor necrosis factor alpha (TNF-α) is essential for protection against tuberculosis despite its role in caseous necrosis exhibited during mycobacterium infection (Flynn et al., 1995). TNF release is induced in macrophages after contact with the pathogen.

IFN release is essential in activating cytokines and the inflammatory response, while CD4 and CD8 T cells are important in protection. CD4 cells produce IFN in response to the presence of Ag85B, a myocolyl-transferase enzyme that is responsible for generating the mycolic acid present on *M. tuberculosis* cell walls (Figure 4) (Cooper & Flynn, 1995). Another key regulator of cytokine release is interleukin (IL)-12. IL-12 release enhances the production of IFN-γ by antigen-specific cells as well as the accumulation of macrophages in infected tissues (Cooper & Flynn, 1995).

II. Host Damage

S. pneumoniae

*S. pneumonia*e colonize the mucosal surfaces of host nasopharynx and throughout the upper airways of the respiratory system, however the pathogen's ability to evade the host immune response renders it capable of spreading into the sterile regions of the lower respiratory tract and causing pneumonia, as seen in Robert (Kadioglu, Weiser, Paton & Andrew 2008). Other manifestations of this disease are acute otitis media (infection of the middle ear), as well as infection in further regions such as the pharynx (Kadioglu, Weiser, Paton & Andrew 2008).

The majority of damaged by *S. pneumoniae* is due to inflammatory tissue injury during infection. For example during complement activation, the release of C3a and C5a causes vasodilation and the release of granule enzymes and toxic oxygen metabolites from phagocytes (Johnston, 1981). *S. pneumoniae* infection also causes necrosis and desquamation of the respiratory epithelium of the tracheobronchial and bronchiolar tree as the bacteria spreads in order to break down the mucociliary barrier to facilitate further infection (Figure 2) (Morens, Taubenberger & Fauci, 2008). Histologically, multiple small abscesses marked with neutrophilic infiltration in airways and alveoli are also often observed in infected patients (Figure 2) (Morens, Taubenberger & Fauci, 2008). Cytokine release is also responsible for triggering fever, as exhibited by Robert.

Infection by *S. pneumoniae* can also be fatal, beginning with acute lung injury due to alveolar epithelial cell injury (Lim et al., 2007). Pneumolysin release by the bacteria has been shown to be associated with the high mortality rates of pneumonia. Research indicates that the direct cytotoxic effect of pneumolysin to the alveolar epithelium and pulmonary endothelium can cause alveolar flooding and haemorrhage (Lim et al., 2007). Subsequently, exudate formation promotes rapid multiplication of *S. pneumoniae* within alveoli, leading to a state known as red hepatisation, where the leakages of erythrocytes into alveoli are observed.

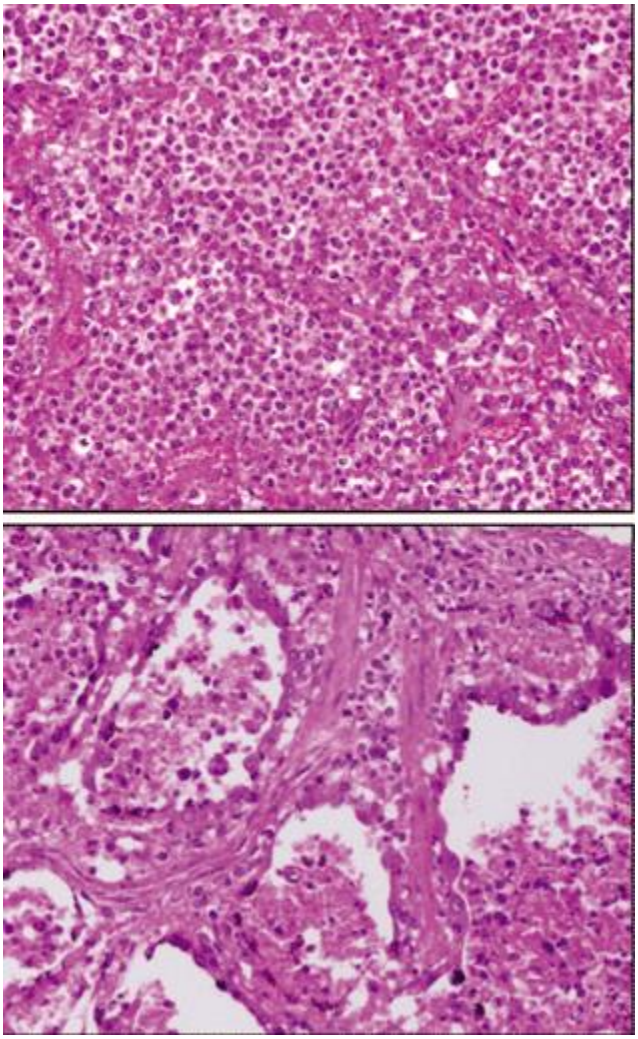

Figure 2. Histology images indicating infection by *S. pneumoniae* leading to neutrophil infiltration in alveoli airspaces (top), and evidence of pulmonary repair and interstitial fibrosis between alveoli (bottom). Image adapted from Morens, Taubenberger & Fauci (2008).

M. tuberculosis

M. tuberculosis causes tissue damage by cell-mediated hypersensitivity (McMurray, 1996). As the bacteria accumulate and the inflammatory response ensues (Figure 3), granulomatous lesions called tubercles develop, which become enveloped by fibroblasts (McMurray, 1996). Further

progression of tubercles leads to caseous necrosis, and eventual calcification of the tubercle (McMurray, 1996). These tubercles play a key role in the persistence of the pathogen, because although they are unable to multiply within the low pH and anoxic environment, they are capable of surviving and remaining dormant for long durations of time, therefore making the pathogen difficult to clear (Todar, n.d.). The persistent nature of the bacteria may explain why Robert has been expressing his symptoms for the past month.

As tubercles grow, they can spread to other parts of the lung, bronchi, and even invade arteries and other components of the circulatory system (Todar, n.d.). Haematogenous spread of *M. tuberculosis* can lead to the development of a condition known as military tuberculosis. If military tuberculosis ensues, secondary lesions and tubercles can form in the genitourinary system, bones, joints, lymph nodes, and peritoneum, leading to further health complications (Todar, n.d.). Eventually tubercles will rupture, releasing large amounts of antigens which cause necrosis and rupture of neighbouring bronchi and subsequent cavity formation (Todar, n.d.). This also allows the bacteria to spread into other airways rapidly.

M. tuberculosis also produce cord factor (a glycolipid) in its cell wall that is toxic to mammalian cells and inhibits polymorphnuclear leukocyte migration (Todar, n.d.). Secreted proteins such as 19-kDA antigenic protein promote the release of cytokines such as IL-12 which activate neutrophils as well as contribute to inflammation in host tissues (Todar n.d.). Inflammation of lung epithelial tissue and accompanied damage can cause the host to experience irritation, therefore leading to repeated coughing bouts as exhibited by Robert.

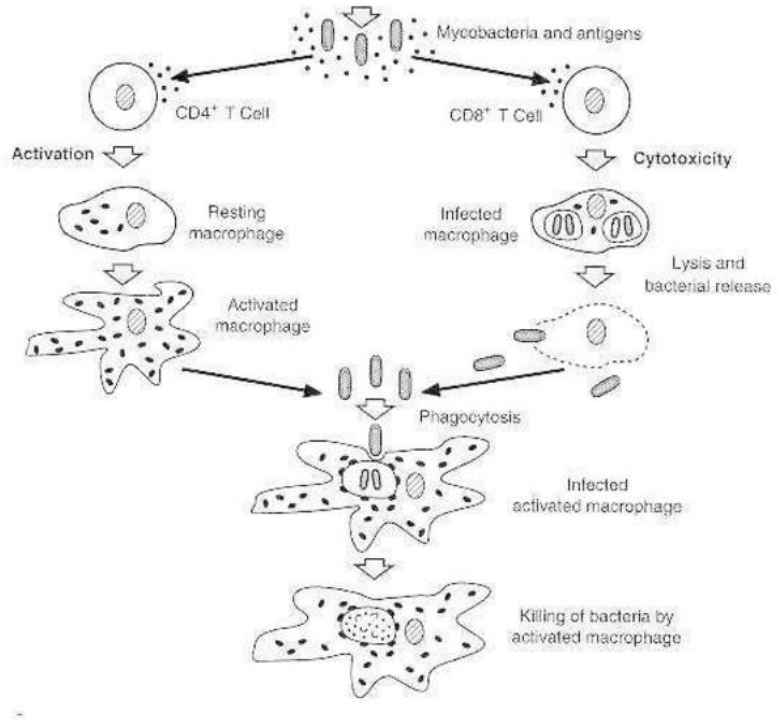

Figure 3. T cell activation and macrophage destruction after infection by *M. tuberculosis*. Image reproduced from McMurray (1996).

III: Bacterial Evasion

S. pneumoniae

S. pneumoniae commonly reside throughout the human respiratory tract as part of the normal flora, often not causing disease unless uncontrolled growth occurs. The polysaccharide capsule protects the bacterium from being phagocytosed by human host macrophages because the antigenic epitopes of the cell wall are close in size and charge to those found on human muscle and connective tissue (Patterson, 1996). In particular, the M proteins are key virulence factors responsible for the bacterium's resistance to the host phagocytic response (Patterson, 1996). PspA proteins (Figure 1) interfere with the host complement cascade, therefore inhibiting complement-mediated opsonisation by the host (Kadioglu, Weiser, Paton & Andrew 2008). Ultimately, these physical features allow *S. pneumonia*e to survive undetected and thus multiply and spread to other locations in the body.

As a Gram-positive bacterium, *S. pneumonia*e cell wall is comprised of techoic acid which leads to polymorphonuclear neutrophil recruitment, changes in permeability and perfusion, cytokine release, and upregulation of platelet-activating factor (Patterson, 1996). After entrance into the nasal cavity, expression of capsule proteins reduces entrapment in the mucus and thus allows the bacteria to access epithelial surfaces in the human host (Kadioglu, Weiser, Paton & Andrew 2008). It is important to note that pneumococcal capsule proteins are negatively charged and thus effectively repulse the sialic acid rich mucopolysaccharides found in mucus. In addition, research suggests that *S. pneumoniae* initially express a thinner capsule during the initial infection phases to maximize adherence and binding to host tissues (Kadioglu, Weiser, Paton & Andrew 2008). Effective adherence to host tissues in the airways is mediated by phosphorylcholine binding to platelet activating factor receptors, which are widely distributed throughout the nasopharynx, due to its similarity to the natural ligand (Kadioglu, Weiser, Paton & Andrew 2008).

Other adhesion tactics employed by *S. pneumoniae* include the production of surface exoglycosidases such as neuraminidase, β-galactosidase, and β-N-acetylglucosaminidase. These exoglycosidases are able to remove the terminal sugar groups of human glycoconjugates and subsequently use them as a nutrient source, or revealing receptors for adherence, or to inhibit the proper functioning of glycosylated host clearance molecules (Kadioglu, Weiser, Paton & Andrew 2008).

The spread of the pathogen to further regions in the human host is facilitated by expression of surface-attached hyaluronidase such as pneumoccoal adhesion and virulence A protein (Pav A) (Kadioglu, Weiser, Paton & Andrew 2008). PavA protein is important for virulence, as its absence has been shown to result in greater host survival. The secretion of zinc metalloprotease limits the host humoral and inflammatory response by targeting and cleaving IgA. This is important during the initial stages of infection because it will allow the pathogen to multiply and colonize, although in later stages the host inflammatory response is upregulated in order to increase mucus production and thus the pathogen's chances of transmission to a new host (Kadioglu, Weiser, Paton & Andrew 2008).

M. tuberculosis

The cell wall of *M. tuberculosis* is comprised of mycolic acids, complex waxes, and a variety of glycolipids (Figure 4) (McMurray 1996). Particular cell wall constituents that contribute to pathogenesis are trehalose dimycolate, mycobacterial sulfolipids, and lipoarabinomannan (LAM). The unique cell wall structure of *M. tuberculosis* protects the bacterium from dehydration, acids, and alkalis (McMurray, 1996), therefore allowing it to survive and adapt to different environments within the human host. During infection, the bacteria reside in alveolar spaces of the lungs and are later engulfed by alveolar macrophages. Many of these bacteria persist intracellularly, therefore remaining undetected by the immune system, where they multiply and eventually kill the macrophage (McMurray. 1996). Intracellular survival is mediated by mechanisms which prevent phagosome-lysosome fusion and acidification by modulating the activity of membrane proton pumps (McMurray 1996). In addition, the abundance of lipids in the cell wall renders the bacteria highly resistant to many antibiotics, therefore limiting clinical treatment options (Todar, n.d.). The fatty acid constituents of the cell wall can also serve as a carbon source for the pathogen during infection (Todar, n.d.). Other consequences of the unique cell wall structure include the bacteria's resistance to death by acidic and alkaline compounds, lysis, and lethal oxidations within macrophages (Todar, n.d.). *M. tuberculosis* counteracts oxidative bursts by producing catalase and superoxide dismutase enzymes which degrade superoxides, and are also capable of downregulating the oxidative cytotoxic mechanism through compounds such as glycolipids, sulfatides, and LAM (Todar, n.d.).

M. tuberculosis bacteria are capable of downregulating the host immune response in order to increase their chances of survival. During infection, protein and mRNA levels of interleukin 1-β, TNFα, and IFN are lowered (Cooper & Flynn, 1995). Depressed immune response is attributed to the reduced antigen presenting ability by mycobacteria-infected macrophages (Cooper & Flynn, 1995). *M. tuberculosis* reduces the expression of B7 co-stimulatory molecule on macrophages which lead to a decreased immune response, in addition to enhancing the production of transforming growth factor (TGF-β) in monocytes (Cooper & Flynn, 1995). The upregulated expression of TGF nullifies the mycobactericial and monocyte recruiting activities of TNF and IFN therefore allowing the bacteria to persist in human hosts (Cooper & Flynn, 1995). Furthermore LAM glycolipids of the cell wall downregulate IFN production as well as inhibit host protein kinase C, thus augmenting the depression of the host immune response (Todar, n.d.).

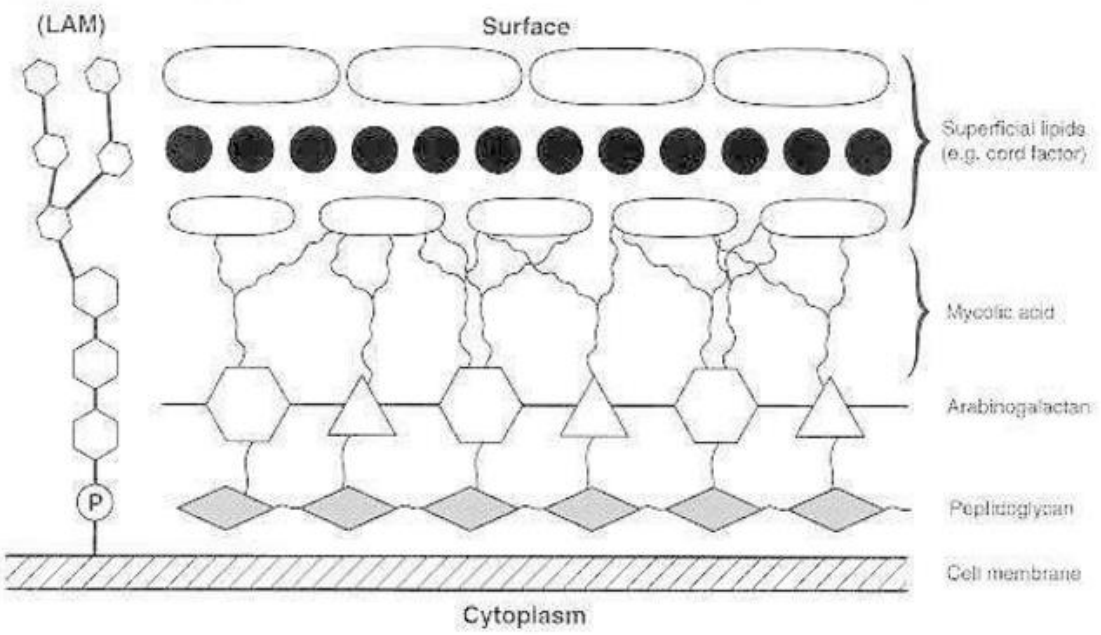

Figure 4. Cell wall composition of *M. tuberculosis*. Image reproduced from McMurray (1996).

IV: Outcome

S. pneumoniae

S. pneumoniae colonies often do not survive during fermentative growth as the colonies age due to the accumulation of peroxide in combination with the lack of catalase and peroxidase. In addition, developing type specific anti-capsular antibodies can allow the host to recover successfully (Patterson, 1996). As discussed in previous sections, infection induces mucosal and systemic immunoglobulin production. Research suggests that pre-existing cross reactive immunoglobulins to PspA is possible, and is related to the decreased susceptibility of individuals to future infections (Kadioglu, Weiser, Paton & Andrew 2008). Other studies suggest that pneumococcal infections primarily occur in individuals that do not possess immunity (such as functional antibodies) to the capsular polysaccharide of *S. pneumoniae* (Musher, 1992). It has been found that most healthy young adults lack antibodies to the majority of capsular polysaccharides, and thus are prone to infection unless they have been vaccinated (Musher, 1982).

M. tuberculosis

M. tuberculosis is a highly contagious pathogen, making it likely for human hosts to be reinfected due to exogenous factors, or experience difficulty in clearing the pathogen if patients have deteriorated immune status (McMurray, 1996). The bacteria are also capable of spreading through the bloodstream to other organs, or reinoculating the lungs causing secondary lung lesions (McMurray, 1996). These lesions often become the location of future infections, which are also attributed to the persistent nature of the bacteria itself. TNF-α release during infection as

part of the host immune response is also important for establishing immunity to the pathogen (Flynn et al., 1995). Although previously infected patients often possess high amounts of *M. tuberculosis* reactive antibodies, there is minimal evidence that indicates that they play key roles in protection (Havlir, Wallis, Boom, Daniel, Chervenak & Ellner, 1991). Furthermore, recent research indicates that the immune response is often unable to achieve sterile clearance of bacilli, and regulatory T cells may actually suppress an otherwise efficient CD4 T cell response. (Kursar et al., 2007).

Clearance of the pathogen and host recovery is highly dependent on an antibiotic regimen that contains multiple drugs due to the high chance of antibiotic-resistant strains of *M. tuberculosis* (Todar, n.d.). By using two or more drugs simultaneously, they help prevent the emergence of tubercle bacilli that are resistant to another drug (Todar, n.d.).

References

Cooper, A. M., & Flynn, J. L. (1995). The protective immune response to Mycobacterium tuberculosis. *Current opinion in immunology*, *7*(4), 512-516.

Flynn, J. L., Goldstein, M. M., Chan, J., Triebold, K. J., Pfeffer, K., Lowenstein, C. J., ... & Bloom, B. R. (1995). Tumor necrosis factor-α is required in the protective immune response against Mycobacterium tuberculosis in mice. *Immunity*, *2*(6), 561-572.

Finger H, von Koenig CHW. Bordetella. In: Baron S, editor. Medical Microbiology. 4th edition. Galveston (TX): University of Texas Medical Branch at Galveston; 1996. Chapter 31. Available from: http://www.ncbi.nlm.nih.gov/books/NBK7813/

Havlir, D. V., Wallis, R. S., Boom, W. H., Daniel, T. M., Chervenak, K. E. I. T. H., & Ellner, J. J. (1991). Human immune response to Mycobacterium tuberculosis antigens. *Infection and immunity*, *59*(2), 665-670.

Johnston, R. B. (1981). The host response to invasion by Streptococcus pneumoniae: protection and the pathogenesis of tissue damage. *Review of Infectious Diseases*, *3*(2), 282-288.

Kadioglu, A., Weiser, J. N., Paton, J. C., & Andrew, P. W. (2008). The role of Streptococcus pneumoniae virulence factors in host respiratory colonization and disease. *Nature Reviews Microbiology*, *6*(4), 288-301.

Koedel, U., Rupprecht, T., Angele, B., Heesemann, J., Wagner, H., Pfister, H. W., & Kirschning, C. J. (2004). MyD88 is required for mounting a robust host immune response to Streptococcus pneumoniae in the CNS. *Brain*, *127*(6), 1437-1445.

Lim, J. H., Stirling, B., Derry, J., Koga, T., Jono, H., Woo, C. H., ... & Xu, H. (2007). Tumor suppressor CYLD regulates acute lung injury in lethal Streptococcus pneumoniae infections. *Immunity*, *27*(2), 349-360.

McMurray DN. Mycobacteria and Nocardia. In: Baron S, editor. Medical Microbiology. 4th edition. Galveston (TX): University of Texas Medical Branch at Galveston; 1996. Chapter 33. Available from: http://www.ncbi.nlm.nih.gov/books/NBK7812/

Musher, D. M. (1992). Infections caused by Streptococcus pneumoniae: clinical spectrum, pathogenesis, immunity, and treatment. *Clinical infectious diseases*, 801-807.

Patterson MJ. Streptococcus. In: Baron S, editor. Medical Microbiology. 4th edition. Galveston (TX): University of Texas Medical Branch at Galveston; 1996. Chapter 13. Available from: http://www.ncbi.nlm.nih.gov/books/NBK7611/

Roe, J. N. (1959). Tuberculosis in Indian immigrants. *Tubercle*, *40*(5), 387-388.

Snapper, C. M., Shen, Y., Khan, A. Q., Colino, J., Zelazowski, P., Mond, J. J., ... & Wu, Z. Q. (2001). Distinct types of T-cell help for the induction of a humoral immune response to Streptococcus pneumoniae. *Trends in immunology*, *22*(6), 308-311.

Todar, K. (n.d.). Mycobacterium tuberculosis and Tuberculosis. Retrieved February 27, 2016, from http://textbookofbacteriology.net/tuberculosis.html

Waites, K. B., & Talkington, D. F. (2004). Mycoplasma pneumoniae and its role as a human pathogen. *Clinical microbiology reviews*, *17*(4), 697-728.