Immunology and Pathogenesis of Pulmonary \textit{Mycobacteria tuberculosis} Infections

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Appendix D - UNIVERSITY HONORS PROGRAM
SENIOR PROJECT - APPROVAL

Name: [Name]
College: Arts & Sciences
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PROJECT TITLE: Immunochemistry and Pathogenesis of Pulmonary Mycobacterium Tuberculosis Infections

I have reviewed this completed senior honors thesis with this student and certify that it is a project commensurate with honors level undergraduate research in this field.

Signed: __________________________, Faculty Mentor
Date: __________

Comments (Optional):
Immunology and Pathogenesis of Pulmonary
*Mycobacteria tuberculosis* Infections

Greg Dunn
Honors Senior Project

Dr. Broadhead
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11/27/01
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I. Abstract

*Mycobacterium tuberculosis* (TB) has long been one of the leading killers worldwide. Approximately one third of the world’s population is infected with tuberculosis with new cases being reported everyday. It is estimated that in 1999 alone there were 3 million deaths as a result of TB. Organisms from *Mycobacterium tuberculosis* are rod-shaped, acid-fast bacilli. On the surface of these bacterium are unique lipids termed mycolic acids. Pulmonary *M. tuberculosis* infections start within the alveolar regions. During an infection these bacterium are able to enter inactive, pulmonary macrophages by way of receptors. Inside these macrophages the bacteria are able to grow and disseminate into other lung tissue. The two factors that decide the outcome of a TB infection are the virulence of the pathogen and the host’s immune response. Less virulent strains of tuberculosis attacking a well-functioning immune system may not result in infection. However, in individuals with weaker immune systems or in cases of highly virulent strains the disease can progress into highly contagious, active tuberculosis. Virulence factors play a major role in TB infections. Such factors as TACO, LAM and hemolysin contribute to mycobacterium’s ability to infect and reproduce within the host. Symptoms of a TB infection are mainly a result of the host immune response. The bacteria doesn’t cause damage to the host tissue, rather the damage is done in the form of necrosis stimulated by the delayed-type hypersensitivity immune response.
II. Introduction

Tuberculosis (TB) has killed more people than any other infectious agent. It has often been called the "White Death" because of the number of lives it has taken over the years. Developing countries are where the highest percentages of deaths occur as a result of TB. The lack of capital, to pay for treatments and for health workers to monitor this disease, is a large obstacle that many third-world countries are faced with in the fight against TB. Because of the complexity and durability of this organism it is difficult to understand and treat. By understanding the mechanisms and behaviors behind a TB infection new treatments can be developed that are more effective and require a smaller duration of treatment.

The recent rise of multiple drug-resistant strains poses a new and more complex problem. The world is currently in a race to find effective methods to control the spread of this ubiquitous pathogen, a race that TB has long been winning. New techniques and initiatives need to be developed to eradicate this disease and eliminate the threat that the world's most deadly infectious agent poses. Through research for new vaccines and treatments against TB, the world can hope to someday overcome this obstacle and focus its research energy on treating and controlling other infectious agents.

III. Methods

Research for this paper was gathered using the internet-based Pubmed database as well as the UT version, Medline. Searches were focused upon virulence factors, entry of TB, drugs, world impact of TB, and the host immune response. The following sections reflect the focus of these searches. Texts were also used to provide both general and
specific information on pulmonary TB infections. Most of the information used was available from multiple review articles. Crosschecking of this information provided assurance that the information was based upon commonly held beliefs instead of isolated supposition. The comparisons made between articles and text should provide information that is relatively accurate.

The initial search came up with specific articles that addressed a very narrow area but were spread over a wide range of topics. The searches were slowly refined until papers, which gave broad overviews of TB infection, were found. These papers were used to decide topics to address. The information used was based upon agreement between more than one source. Any ideas that weren't supported by a general consensus or opinions that were irrelevant were not used.

IV. Body

A. Overview of Tuberculosis infection

*M. tuberculosis* enters the body through inhalation of the pathogen and the subsequent infection of pulmonary macrophages. Once inside the lung, macrophage receptors recognize the mycobacterium and take up the pathogen into an immature phagosome. Typical pathogens that enter macrophages would be normally degraded in lysosomes. Within *M. tuberculosis*, in the context of active infection, this does not occur. The mycobacteria are able to resist lysosomal degradation and even multiply within the macrophage phagosome. There are two factors that influence the outcome of an infection with *M. tuberculosis*: the virulence of the pathogen and the host response to the infection. Because these two factors have different effects in different circumstances, the range and outcome of tuberculosis infection varies greatly. Less virulent strains of tuberculosis
attacking a well-functioning immune system may not result in infection. In a case like this, the bacteria are either killed or go into a dormant state in which disease progression does not occur. In individuals with weaker immune systems or in cases of highly virulent strains the disease can progress into highly contagious, active tuberculosis.

In the first case, a host macrophage phagocytoses the pathogen and is able to either kill the pathogen or contain it within granulomas. Granulomas are lymphocyte-surrounded lesions that contain macrophages and \textit{M.tuberculosis} bacilli. These granulomas control growth by creating an anoxic environment in which the pathogen cannot reproduce. This environment stimulates the pathogen to shut down its major metabolic pathways and enter into a state of dormancy called nonreplication persistence (NRP) (1). The bacilli stay dormant for several years and can only be reactivated if the host’s immune system is compromised. In infected individuals with this type of passive infection there are no symptoms of disease and the infection is not contagious.

Active TB infections have completely different symptoms than passive TB infections. In active TB the bacilli can still enter macrophages but the macrophage is unable to control the growth of the pathogen. The ability of the macrophage appears to be dependent upon the state of activation of effector cells such as CD4+ helper T-cells (2). Eventually lysis of the macrophage occurs and the pathogen is released to the extracellular environment and is able to infect other cells. The ability to control the proliferation of \textit{M.tuberculosis} within the macrophage is the critical difference between active and passive TB infection.
B. Characteristics of Mycobacterium

Organisms from the genus *Mycobacterium* consist of rod-shaped, acid-fast bacilli.

On the surface of mycobacterium are unique lipids termed mycolic acids. Mycolic acids have played a significant role in the identification of members of this genus (3,4).

Mycolic acids are attached to peptidoglycan on the cell wall which gives the cell a waxy surface with low-permeability. This waxy surface is important for survival of Mycobacteria, but it is also a cause for the slow growth of *Mycobacterium tuberculosis* (*Mtbb*). The low permeability of mycobacteria prevent the organism from being easily classified by the Gram stain. To classify Mycobacterium according to the Gram method it is necessary to remove the waxy surface with either alkaline ethanol (3) or by melting the wax barrier surrounding the cell wall (5). Once this is done, the remaining cell stains Gram positive.

On solid media *M.tuberculosis* shows growth that results in tight, wrinkled colonies that clump in their growth patterns. It is thought that the hydrophobic nature of the bacilli’s cell wall contributes to this unusual pattern (3). *M.tuberculosis* requires days to weeks of incubation before a culture will have large enough colonies to work with.

Egg-yolk is typically used in the culturing of mycobacteria because it is a source of lipids that the bacilli can make use of within its cell wall (3). *M.tuberculosis* was first extensively studied by Robert Koch and was one of the first studies to apply the four
criteria of Koch's postulates. One of the more unique properties of mycobacteria is their generation of yellow carotenoid pigments. *M.*tuberculosis create these pigments when the organism is cultured in light. Carotenoid pigment formation requires short-wavelength light and O$_2$ (3). The probable use of carotenoid is in the protection of the mycobacteria from oxidative damage by singlet O$_2$ formation (3). Virulence within *M.*tuberculosis has been associated with cord factor. This glycolipid causes the formation of long, cordlike structures when grown on agar medium (3). The cord shape is a result of the intertwining of long chains of bacilli.

**C. Host Immune Response**

The body's response to *M.*tuberculosis is critical to the infection process. Damage done to host tissue is typically self-induced. Much of this damage is from such events as the formation of granulomas, sustained macrophage activation and fibrosis of lung tissue. All of these events are methods that the immune system uses to contain the bacteria but these eventually lead to significant host damage. The host response to an *M.*tuberculosis infection occurs in a cycle that, in active tuberculosis, is perpetuated until either the disease is eliminated or the death of the host occurs.

1. Host-cell entry

Receptor-ligand interactions play an important role in the entry of pathogen into the host macrophage. The type of receptor used in uptake is also thought to be crucial in whether a diseased state occurs. Two types of entry are possible into the macrophage: an uptake that results in productive infection vs. an uptake that leads to an abortive invasion (6). With these two types of receptor-mediated uptake, macrophages can serve as either a
refuge for the pathogen or as its killer. Which route the infection takes within the macrophage is thought to be dependent upon which receptors are involved in the uptake of *M. tuberculosis*.

Although still up for debate, several have considered Fc receptors to be responsible for abortive invasion by *M. tuberculosis* (6). In this suggested mechanism FcγRII receptors recognize antibody-opsonized pathogen. After recognition and uptake of the mycobacterium by way of these receptors the macrophage attacks the pathogen with reactive oxygen and nitrogen intermediates (6). This attack has lead to continued research into whether receptor type is a determining factor in pathogen survival. In some studies it was found that the viability of bacteria taken up by Fc receptors is significantly decreased, while other studies have shown that the viability after uptake is the same. Complement and mannose receptors are largely responsible for entry within productive infections. Mycobacteria contain both LAM and a phenolic glycolipid that are able to bind to the thioester bond on complement factor C3 (6). This binding of C3 to the surface of M. tuberculosis provides for rapid uptake of the pathogen into the macrophage by way of complement receptors 1, 2 and 3 (7). These receptors do not trigger strong microbicidal behavior which allow for entry of the pathogen but do not cause its destruction (6).

2. **Intracellular control of infection**

The ability of *M. tuberculosis* to establish an intracellular infection is dependent upon the acidification level of its phagosome and the fusion of lysosomes. The activation level of the macrophage serves as the most important determinant in whether an infection
is controlled. Higher levels of activation within the macrophage create endosomal environments less favorable for *M. tuberculosis* growth. If the endosome maturation is halted at an early stage then the mycobacteria will not be attacked by enzymes, acid, intensive ROI and RNI attacks, and the mycobacteria will still have access to nutrients, of which iron is the most essential. *M. tuberculosis* is able to halt the phagosome maturation through such virulence factors as TACO, which is thought to prevent lysosome fusion by preventing microtubule formation on the phagosome.

Two types of responses can take place within sites of *M. tuberculosis* infection. Cell-mediated immunity (CMI) is beneficial in eliminating the infection without damaging host tissue. This response expands the number of T-cells within the infection site that are able to produce cytokines. The important cytokines produced by T-cells include interferon-γ (IFN-γ) and tumor necrosis factor-α (TNF-α) (6,8). IFN-γ is able to bind to macrophage and cause autocrine stimulation by way of interleukin-12 (IL-12) (8). An effective CMI response is the ideal way to have a TB infection controlled. The bacilli are ingested and killed by macrophages without extensive necrosis of pulmonary tissue.
The other possible immune response, delayed-type hypersensitivity (DTH), is destructive to host tissue and is responsible for the symptoms of tuberculosis. Although controlled by the same Th1-type T-cells as CMI, DTH responses result in extensive tissue necrosis and scar tissue formation (8). The difference is seen when the levels of bacterial antigens reach high levels within the infection site. This high level of stimulation by these antigens causes the death of unstimulated, bacilli-laden macrophages and the surrounding tissue. Much of this damage can be attributed to stimulation by CD8 cytotoxic T-cells (9,10). The initial immune response is typically a delayed-type hypersensitivity because the majority of macrophages are in an inactive state (8). The caseation of the tissue controls growth until activated macrophages are able to intervene and stop infection.

The area in which an infection and immune response occurs is commonly known as a tubercle or in cases of advanced infection, a granuloma. This area consists of inactive macrophages in the center, surrounded by activated macrophages and lymphocytes (2,11). On the outside of these activated cells is another group of naïve, inactive macrophages that have been attracted by way of chemotaxis to the site of infection (2). Sometimes mycobacteria can escape the tubercle and infect these outer macrophages. When this occurs a new tubercle is developed and the CMI must be reactivated. This spread of the bacterium out of the tubercles is responsible for the pulmonary spread of tuberculosis (8). For the body to control infection it must be able to contain the infection and not allow dissemination of the bacilli. When the immune system is unable to kill the pathogen within the center of the tubercle then fusion of the inactivated, bacilli-laden macrophages occur resulting in the formation of a granuloma
(12). Tissue death around this granuloma occurs creates an anoxic, dry environment (12). This unfavorable environment signals the bacilli to go into a dormant non-reproductive state in which further spread of bacilli is prevented. Unless stimulated by heat and oxygen, the bacilli can stay in this low metabolic state indefinitely (1,13).

3. Effector mechanisms

Developed resistance to TB infection depends upon Th1-cell mobilization. Th1 cells are extremely important within CMI immune responses. These cells produce IFN-γ, IL-2 and lymphotoxin, which are essential in activating the microbicidal activity of macrophages (2,11).

Mycobacterium-laden macrophages release TNF-α, and IL-12, which stimulate Th1 helper cells and natural killer (NK) cells and attract new lymphocytes to the site of infection by way of chemotaxis (6,11). TNF-α serves both autocrine and paracrine activating functions while IL-12 is solely paracrine (2). After stimulation by IL-12, NK and Th1 cells releases IFN-γ, which significantly increases the activation level of the macrophage (11). After the initial infection, macrophages also secrete IL-1, IL-3, IL-6 and macrophage chemoattractant protein 1 (MCP-1) (6). These cytokines chemotactically recruit lymphocytes and naïve macrophages to the site of infection. After an infection is
controlled, the macrophage release IL-10 which self-inactivates the macrophage as well as surrounding lymphocytes and NK cells (2).

The methods in which the various lymphocytes and monocytes work together to control infection is a complex process. Some of these cells have been found to be important in controlling M. tuberculosis but the contribution they make to the immune response is still unclear. γ/δ T-lymphocytes and CD8 cytotoxic T-cells have been found to play an important role in the control of tuberculosis infections (8,11). γ/δ T-lymphocytes are known to recognize non-protein antigens that do not have to be displayed by the major histocompatibility complex (MHC) (8). One of the more important antigens that these lymphocytes recognize is cord factor (trehalose dimycolate) (8). These lymphocytes also appear to be important in resistance to clinical tuberculosis among tuberculin-positive health care workers (8).

The role that CD8 T-cells play in a tuberculosis infection is also important but not as critical as that of Th1 cells. Within the murine model, CD8-KO mice were unable to control the infection resulting in eventual death of the mice (9). Turner et al. have shown that CD8 T-cells use the CD95/CD95L (Fas-FasL) pathway to trigger the infected macrophage to go into apoptosis (9). It is believed that the apoptotic death mediated by CD8 T-cells serves to release viable bacteria from inactive macrophages and allow for the pathogens uptake into active macrophages (8,9,10). This release and subsequent uptake prevents uncontrolled proliferation of the bacilli within macrophages (10). Extensive DTH activation of CD8 T-cells causes much of the damage to host tissue. The extended presence of lymphocytes, neutrophils and macrophages cause damage to host tissue through the cytokines produced. Another lymphocyte thought to be important
within a TB infection is CD1-restricted T-cells. The exact role these cells serve is unclear, however they are thought to be involved in recognizing glycolipids that are expressed on *M.tuberculosis* while it is in a phagosome (14).

**D. Virulence factors of *M.tuberculosis***

Unlike the majority of pathogenic bacteria, tuberculosis does not contain a toxin that causes damage within host tissue. Instead the destruction comes from the host immune response. Although there is no toxin, *M.tuberculosis* does contain several virulence factors that help the bacterium survive. The majority of these virulence factors are involved with entry of the pathogen into host macrophages and the proliferation of the mycobacteria once inside this host tissue. One studied virulence factor is the recruitment of a 50kDa phagosome coat protein TACO (tryptophan aspartate-containing coat protein) (15). This protein was characterized and analyzed extensively in 1999 and is found on the membranes of phagosomes containing pathogen (15). Endosomes that don’t contain this protein are able to mature into or bind to mycobacterium-killing lysosomes (15,16). These lysosomes kill the bacteria through enzymatic and acidic degradation and are essential in host immune defenses. When TACO is present within the phagosome membrane, the membrane is unable to mature and bind to cellular lysosomes (15,16).

TACO is a member of the family of WD repeat proteins and is produced within host tissue (15). The WD proteins are have not been found within prokaryotes and are typically involved in signal transduction, motility, cytoskeletal organization and vesicle fusion (15). It is thought that TACO plays a role in tubulin recruitment within the host cell. This interaction implies that TACO is involved in microtubule formation (15).
Within cells not infected with *M. tuberculosis*, TACO is probably a signal to prevent lysosomal binding (15). The mechanism by which TACO is recruited by *M. tuberculosis* is unknown, however it is thought that the interaction with *M. tuberculosis*-containing phagosomes occurs through association with cholesterol (16). One interesting fact is that TACO is not present within Kupffer cells in the liver. It appears that these liver cells naturally down-regulate TACO, which may partially explain why the liver is the site of mycobacterium removal in the body (15).

Another virulence factor important in *M. tuberculosis* infections is involved in entry of mycobacteria into the host macrophage. Phagocytic uptake into macrophages requires the binding of pathogen to macrophage receptors. These receptors include complement receptors, mannose receptors, scavenger receptors and Fc receptors that take-up antibody bound bacteria (17). It appears that mycobacteria contain a receptor that is capable of binding cholesterol found within macrophage cell membranes. When cholesterol is removed from macrophages, it has been found that *M. tuberculosis* is unable to enter into host macrophages (16). Once these initial interactions take place then other macrophage receptors interact and take-up the bacterium (16).
There appears to be another factor at work that remains undefined but active within mycobacterial phagosomes. This factor blocks the transport and maturation steps between early and late endosomes. Within these phagosomes expression of rab7 is not seen. Rab7 is a GTPase that is found in late endosomes and is suspected of regulating traffic within the membranes of mature endosomes (16). Rab5 expression is typically found to indicate lack of rab7 and is typically associated with early endosomes (16). This protein is indeed found within phagosomes that contain live, propagating mycobacteria (16). Whether TACO or some other factor is responsible for the blocking of rab7 expression is to be determined, however it is apparent that the factor responsible for this activity is essential in M. tuberculosis virulence. Limited acidification of the endosome is also seen within phagosomes (16). This low acidification is due to an unknown or undefined M. tuberculosis virulence factor that prevents the production of the proton-ATPase responsible for the acidification of the phagosome.

The mycobacterial cell envelope largely consists of a mycolyl arabinogalactan-peptidoglycan complex and associated lipoarabinomannan (LAM), a glycolipid. It is thought that the lipids within the cell envelope mediate specific interactions with host ligands or membranes and serve as lesser virulence factors (4). It is suggested that one of the reasons for LAMs importance in the cell is that it prevents macrophage and leukocyte activation (18). How this is done is unknown, but tests comparing lipoarabinomannan types indicate variations in the levels of activation. M. tuberculosis LAM appears to elicit a chemotactic response but whether this is important or not is unknown. It is thought that the response of T-cells to the site of infection might allow the pathogen to signal the T-cells into a state of anergy (deactivation) (18). Unfortunately this is just speculation since
the real mechanism and purpose is unknown. Another interesting trait of bacteria containing LAM is that the chemotactic, granulocytic response was not present (18). A lack of neutrophils was found within the strains of *M. tuberculosis* that contained the virulent form of LAM (18). A final trait of LAM is that it might prevent the production of reactive oxygen intermediates (11). This ability combined with the reduced acidification present within the phagosome would help prevent *M. tuberculosis* destruction.

A final virulence factor is hemolysin (11,19). This factor is thought to be responsible for lysing the phagosome containing the pathogen. This releases the *M. tuberculosis* into the cytoplasm of the macrophage where lysosomes and other factors cannot contribute to the destruction of the pathogen (11,20,21). Without the presence of lysosomal fusion, acidification, or superoxide attack *M. tuberculosis* is able to freely divide and is eventually released from the host macrophage. It is unclear how important this factor is to overall infection but if *M. tuberculosis* is able to move into the cytoplasm, uncontrolled population expansion can easily take place.

**E. Current treatments and vaccines against tuberculosis**

Vaccines and antibiotics that are effective against TB have been very difficult to find. The difficulty in finding vaccines is that antigens that stimulate an effective antibody response haven’t been found. Drugs are difficult to fine because mycobacteria have both inherent and conferred resistance to almost every single antibiotic. The inherent resistance is due to both the mycolic acid surface as well as the difficulty of diffusion through a fibrous granuloma or tubercle. There are four populations that bacilli
might fall under: active and extracellular within cavities, in closed tubercles, within macrophages and dormant. Because no antibiotic is effective against all four of these populations, treatment that makes use of a cocktail of antibiotics is necessary. These factors combine to make the eradication of Mtb a daunting task.

I. Vaccines

BCG (bacillus Calmette-Guérin) is currently the only vaccine available against tuberculosis. Calmette and Guérin in 1906 originally observed TB resistance when pigs where inoculated with an equine strain of Mycobacteria. After modification of a bovine strain they were finally able to come up with the BCG vaccine that was recommended, in 1928, by the League of Nations for widespread use (21). The BCG vaccine varies in immune protection from 0% to 80% depending upon which strain is involved (20). This level of efficacy is too low to have much of an effect in controlling the spread. BCG only delayed the spread of TB for a few years by selecting against the strains that BCG was effective against. The countries that have the greatest burden from tuberculosis are the ones in which the BCG vaccine is least effective (20).

More effective vaccines need to be developed against TB. One type of vaccine that has been considered uses a recombinant BCG strain (14,20,21). This strain has a gene for hemolysin, the protein that allows M.tuberculosis to escape into the cytoplasm (20,21). Another recombinant BCG strain that expressed Ag85A/B/C in high amounts increased the efficacy of the vaccine (20). Other antigens to place within recombinant strains are being looked at but very few have been found that increase resistance to TB.
Two other approaches to vaccines include protein subunit vaccines and DNA vaccines (19,20,21). Subunit vaccines use proteins that stimulate an immune response. Two of the subunit vaccine types being considered are secreted proteins and stress proteins that are synthesized by the bacteria during the later stages of infection (19). The subunit approach requires that antigens which produce an immune response are located and presented to the immune system in a way that stimulates a response (14). The subunit approach is preferred in many ways because the chance of vaccine-related illness occurring is greatly reduced. DNA vaccines are also being considered. By inserting DNA that expresses mycobacterial antigens into a retrovirus, the production of the antigens allow for an immune response to be initiated (19). This approach is favorable to the subunit method because there aren’t purification and packaging problems that are inherent with subunit vaccines (19). Both hemolysin and Ag85 are antigens that can be used within subunit and DNA vaccines. If a subunit or DNA vaccine is eventually developed then it is likely that several antigens or their genes will be expressed or present within the vaccine.

2. Chemotherapy

Isonazid, rifampin and pyrazinamide have been the front-line, anti-tuberculosis drugs since the 1950s (22). All three of these drugs have Minimal Inhibitory Concentrations (MICs) that are close to the concentration in which they become toxic to host tissue (22). This shows the ineffectiveness of these drugs and emphasizes the need for more effective treatments. These three drugs are the only bactericidal, front-line treatments (23). These three drugs are all prodrugs that become effective when they are
modified by *M. tuberculosis* enzymes (22). Unfortunately the rise of drug resistant TB strains occurs quickly in the presence of prodrugs. This attribute is seen because one mutation in the gene responsible for converting the drugs into their active form is all that is needed to confer resistance. Two other drugs, ethambutol and streptomycin, are considered front-line drugs (23). These are bacteriostatic and are effective in complementing the immune system or the other first-line drugs.

Very little is known of the targets and activity of many of the front-line anti-TB drugs. Studies of isonazid found that after treatment with INH the specimens lost their acid-fastness (24). This implies that isonazid interferes with mycolic acid synthesis thereby hindering the biosynthesis of the bacterial cell wall (24,25). Point mutations within the *inhA* gene confer mycobacterial resistance to isonazid (24). Rifampin is known for its rapid diffusion across the mycobacterial cell envelope and its activity against RNA polymerase (24). Mutations in the gene coding for the β-subunit of RNA polymerase frequently confers resistance to rifampin (24). The mode of action for the third, front-line anti-TB drug, pyrazinamide, is unknown. Resistance to this gene is conferred when mutations in the *pncA* gene are present. This drug is active against bacilli in low pH environments (such as a phagosome) after it is converted to pyrazinoic acid (24). The two, less important first-line drugs, ethambutol and streptomycin, have bacteriostatic modes of action. Ethambutol is thought to interfere with glucose incorporation into cell wall polymers, while streptomycin is thought to interfere with the transition of bacterial ribosomes from the initiation complex to the elongation complex (25).
The current repertoire of TB drugs is very limited and with the rise in MDR strains of TB, new drugs need to be developed. There are several different approaches to finding new drugs. Modification of the current front-line and second-line TB drugs shows promise, although in many cases the bacterial resistance against these drugs is preserved (24). The search for new antibiotics might also yield a new drug that is effective against TB. A third method of finding new anti-TB drugs might make use of computer protein modeling to determine drug-pathogen interactions (24). It has been found that, in general, the more hydrophobic an antibiotic is, the greater its potency (22). Another factor that would help in the development of new antibiotics is finding drugs that do not require modification by the mycobacterium before they become effective. This attribute would prevent the bacterium from developing immunity as quickly and as effectively (22).

F. World strategies and initiatives against Tuberculosis

*Mycobacterium tuberculosis* (TB) has long been one of the leading killers worldwide. Approximately one third of the world’s population is infected, with new cases being reported everyday. It is estimated that in 1999 alone there were 8.4 million new cases (26). This was an increase from the estimated 8.0 million new cases estimated in 1997 (26). Tuberculosis isn’t just a problem within developing countries. The United States has seen a sharp increase of cases due to lagging public policy as well as the increase in cases of MDR (Multi-drug resistant) TB strains (27). These MDR resistant strains can only be treated with less effective antibiotics that the bacilli haven’t developed resistance to. More effective antibiotics are needed to treat these new strains.
In 1992 the WHO (World Health Organization) declared TB a global emergency, an unprecedented step. Initiatives have since been adopted by the WHO that call for the reduction and eventual elimination of TB. These initiatives call for detection of 70% of new cases of TB and the effective treatment of 85% of these cases by the year 2005 (26). Unfortunately the WHO is a long way away from these objectives. Instead of reaching these objectives by 2005 it is estimated that these goals won't be reached until 2013 (26).

DOTS (Directly Observed Treatment, Short-course) is the strategy recommended by the WHO to help reach these goals TB. DOTS consists of five key components (26):

- **Government commitment** to sustained TB control activities.
- **Case detection by sputum smear microscopy** among symptomatic patients self-reporting to health services.
- **Standardized treatment regimen of six to eight months** for at least all sputum smear positive cases, with directly observed therapy (DOT) for at least the initial two months.
- **A regular, uninterrupted supply of all essential anti-TB drugs.**
- **A standardized recording and reporting system** that allows assessment of treatment results for each patient and of the TB control program performance overall.

*Taken from: The World Health Report, 2001*

The DOTS method of treatment calls for individually tailored treatment of TB. This is necessary because of the ineffectiveness of antibiotics on MDR strains. In extreme cases in which the drugs available for treatment are ineffective, the infected section of the lung has to be surgically removed. The complexity inherent in treating patients with MDR requires significant human resources. This cost is necessary because failure to reduce worldwide TB infection could see the rise in new MDR strains that are easily spread and cannot be treated. The WHO compares this possibility as having a threat akin to the HIV and Ebola viruses.
V. Summary

Pulmonary tuberculosis infections rely on a unique balance between the host immune response and the virulence factors present within the pathogen. Survival in a pulmonary macrophage requires that such virulence factors as TACO recruitment and LAM be expressed by the bacterium. *M. tuberculosis* prevents its destruction within host macrophages by preventing lysosome-phagosome fusion and by preventing acidification of the endosome. The waxy mycolic acids surrounding the pathogen also serve many functions that prevent the destruction of the pathogen within macrophages. Control of TB infections require that macrophages be activated by Th1 helper T-cells and by natural killer cells. This activation is controlled by the release and uptake of cytokines essential in activation of the CMI and DTH immune responses.

Tuberculosis has long been a complex and devastating world problem. The lack of initiative, consistency and discipline in monitoring and treating tuberculosis has lead to a state of near uncontrolled spread of TB. World initiatives need to be adhered to by each country to help control the spread of this disease. New vaccines, effective in developing a host immune response against sustained TB infections, would greatly reduce the impact this disease has upon the world. New drugs also need to be developed that provide effective treatment against the MDR strains that have arisen. Increases in public sector spending on TB research are the best way to develop these new therapies. Because TB is largely a third world disease, any new therapies developed would not have a great capital demand. This lack of capital necessitates public-sector development since pharmaceutical companies will not research a disease in which little to no profit could be realized.
VI. References


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