Mycobacterium smegmatis: A Model Organism for Mycobacterial Research

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Mycobacterium smegmatis is an acid-fast bacterial species in the phylum *Actinobacteria* and the genus *Mycobacterium*. It is Gram positive soil bacteria of 3.0 to 5.0 µm long with a bacillus shape, seldom causes any infection to human. *M. smegmatis* is a highly attractive surrogate host for the analysis of mycobacterial genes can be grown in common media like Luria-Bertani, nutrient and tryptone broth etc. The *M. smegmatis* strain mc^2155 has proven to be a work house for the molecular analysis of genes from mycobacteria and mycobactriophages. It is fast growing, nonpathogenic readily transformable (Snapper *et al.,* 1990) with integration proficient plasmids and pAL5000 based episomal plasmids, and it readily undergoes homologous recombination, allowing for the generation of targeted gene disruption. Similar antibiotic responses and other physiological similarities with *M. tuberculosis* (causative agent of pulmonary tuberculosis) make it appropriate model for studying the biology of mycobacteria.

History and epidemiology of tuberculosis

Consumption, King's Evil, Lupus Vulgaris, and Phthisis are some of the more colorful names for tuberculosis (TB) that have been used in the last several centuries. The disease traces its origins to antiquity when man first domesticated and M. tuberculosis evolved from M. bovis (Daniel *et al.,* 1994). The history suggests that tuberculosis was a rare disease among early nomadic human populations but became endemic among settled populations 15000 years ago (Sreevatsan *et al.,* 1997). Mummified human remains from Egypt show evidences of TB, including characteristics skeletal deformities and fibrotic lung lesions containing M. tuberculosis DNA (Crubezy *et al.,* 1998). The rise of the cities with dense populations provided ideal conditions for transmission of the air borne pathogen. Society flourished and trade routes became a mechanism for the spread of disease into new areas.

The TB epidemic reached a peak during the 19th century when it became the leading cause of death in the western world. The overcrowded unsanitary cities of the industrial revolution were devastated, especially within the poorer sections of the cities with malnourished populations. By this time the disease was pervasive throughout society and was no longer a disregarded scourge of the poor. In artistic circles TB became a romantically tragic disease, and it was the subject of works of art, literature and music. By the end of the 19th century the incidence of tuberculosis was steadily declining. Many reasons are cited, including the Pasteur's discovery

of microbes as a cause of disease, but perhaps most important was an improvement in the standard of living in many countries (WHO Report of tuberculosis epidemic 1997). Robert Koch famously identified Mycobacterium tuberculosis as the organism that causes TB in 1882. The world press hailed an imminent cure, but an effective treatment remained elusive for over 70 years. While the disease could be diagnosed with accuracy by 1905, largely due to Wilhelm Konrad Rontgen's discovery of X-rays in 1895 and Koch's stain for microscopy, nothing could be done to cure the disease.

The first effective treatment of TB was discovered by Selman Waxman at Rutgers University in 1939. Waxman observed that certain soil dwelling organisms had an inhibitory effect on mycobacterial growth and coined the term "antibiotic". His laboratory subsequently isolated streptomycin from Streptomyces griseus, and Waxman was awarded the 1945 Nobel Prize in medicine. On November 20, 1944 the first human subject received streptomycin: Patricia, a young Minnesotan woman who was near death from progressive pulmonary tuberculosis. Other compounds rapidly followed the discovery of streptomycin, including p-amino salicylic acid (1949), isoniazid (1952), pyrazinamide (1954), cycloserine (1955), ethambutol (1962) and rifampin (1963). The advent of multidrug therapy dropped the rate of TB infection precipitously in the latter half of the 20th century.

The global distribution of TB cases at present is skewed heavily towards low-income and emerging economies (WHO report 2008). The highest prevalence of cases is in Asia, where China, India, Bangladesh, Indonesia, and Pakistan collectively make up over 50% of the global burden. Africa, and more specifically sub-Saharan Africa, has the highest incidence rate of TB, with approximately 83 and 290 per 100,000, respectively. TB cases occur predominantly (approximately 6 million of the 8 million) in the economically most productive 15- to 49-year-old age group.

In 2006, around 14.6 million people had active TB disease with 9 million new cases. The annual incidence rate varies from 356 per 100,000 in Africa to 41 per 100,000 in the Americas. TB has also now become the world's greatest infectious killer of women of reproductive age and the leading cause of death among people with HIV/AIDS. The emergence of HIV infection, especially within incarcerated populations such as prisons, hospitals and homeless shelters lead to outbreak of infection (Gandhi *et al.,* 2006). The rise in HIV infections and the neglect of TB control programs have enabled a resurgence of tuberculosis.

The emergence of drug-resistant strains has also contributed to this new epidemic with, a large number of TB cases being resistant to standard treatments and resistant to second-line drugs. The rate at which new TB cases occur varies widely, even in neighboring countries, apparently because of differences in health care systems.

Treatment of Tuberculosis

In the middle of 20th century, the outlook of TB patients and the history of TB dramatically changed with the introduction of chemotherapy. The discovery of p-amino salicylic acid (PAS) by Jorgen Lehmann and of Thiosemicarbazone by Behard Domagk during World War I yielded the first therapeutic agents with efficacy in the treatment of TB. In 1944, Albert Schatz, Elizabeth Bugie and Selman Waksman reported the isolation of streptomycin, the first antibiotic and first bactericidal agent effective against *M. tuberculosis* (Schatz *et al.,* 1944). With the discovery of isoniazid, the first oral drug in 1952, and rifampin in 1957, a new chemotherapeutic era of TB treatment had dawned and sanatoria were closed. The discovery of the two effective drugs with their application in the armamentarium of anti-tuberculosis strategy in 1966 (Youatt *et al.,* 1969) accelerated investigation on reduction of tuberculosis treatment. In the middle of 1970s, comparative studies of different combinations of these drugs demonstrated that the 6-months treating regimens containing rifampin or pyrazinamide showed superior curing efficiency for patients. Isoniazid and rifampin were considered as complete bactericidal drugs, being capable of killing bacteria in all environments, while streptomycin and pyrazinamide were of 'half' effective, the former being active in the more alkaline milieu and latter active in acidic intracellular environment. Thus, the combination with streptomycin or pyrazinamide was considered as perfect complementary combination which made a very powerful bactericidal regimen (Fox *et al.,* 1975). Therefore, the short course regimen began to employ streptomycin, isoniazid and rifampin, which suggested that at least two full bactericidal drugs were required to successfully and dramatically cut treatment duration from 12-18 months down to 6 months.

Drug Used in Treatment of Tuberculosis:

Agent	**Mechanism of Action**
Rifampicin (RIF)	Inhibits bacterial RNA synthesis by binding to the β subunit of bacterial DNA-dependent

	RNApolymerase (DDRP) Inhibition of DDRP leads to blocking of the initiation chain formation in RNA synthesis. One of the most effective antituberculosis agents available and is bactericidal for intra- and extra-cellular bacteria.
Isoniazid (INH)	Most active drug for the treatment of TB caused by susceptibe strains. Is a pro-drug activated by katG, which exerts its lethal effect through inhibition of synthesis of mycolic acids, an essential component of mycobacterial cell walls, through formation of a covalent complex with an acyl carrier protein (AcpM) and KasA, a betaktoacyl carrier protein synthetase.
Pyrazinamide (PZA)	Converted to the active pyrazanoic acid (encoded by pncA) by pyrazinamidase in susceptible organisms. Pyrazanoic acid lowers pH in then immediate surroundings of *M. tuberculosis* – organism is unable to grow. May also function as an antimetabolite of nicotinamide and interfere with the synthesis of NAD, inhibiting the synthesis of short-chain, fatty-acid precursors.
Ethambutol (ETB)	Inhibits mycobacterial arabinosyl transferases (encoded by the embCAB operon) involved in the polymerization of D-arabinofuranose to arabinoglycan, an essential cell wall component.
Streptomycin, kanamycin, amikacin, capreomycin	The aminoglycosides are irreversible inhibitors of protein synthesis through binding to specific 30Ssubunit ribosomal proteins.
Ciprofloxacin, ofloxacin, levofloxacin, moxifloxacin, gatifloxacin, sparfloxacin	Inhibit bacterial DNA synthesis through inhibition of bacterial topoisomerase II (DNA gyrase) and topoisomerase IV, which are responsible for the relaxation of supercoiled DNA and the separation of replicated chromosomal DNA, respectively.

Ethionamide	Chemically related to INH, converted via oxidation to ethionamide sulfoxide, blocks the synthesis of mycolic acids.
Cycloserine	Structural analogue of D-alanine, inhibits incorporation of D-alanine into peptidoglycan pentapeptide through inhibition of alanine racemase.

Table: Classes of Anti TB drugs and their mode of action (*Respiratory Research* 2006)

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