II. LITERATURE REVIEWS

A. Tuberculosis (TB)

Tuberculosis is an ancient and venerable infectious disease that still afflicts both men and domestic animal throughout the world. The etiologic agent of the disease is the bacteria known as *Mycobacterium tuberculosis*. Tuberculosis may affect any part of the body. The most commom form occurs at the lungs, which leading to pulmonary tuberculosis. The commonest non-respiratory sites are the lymph nodes of the neck and supraclavicular region, abscesses at any site, bone and joint disease, particulary of the spine, genitourinary disease, abdominal and peritoneal disease, pericardial disease and meningeal and central nervous system (CNS) disease. Symptoms depend on the site involved, but are usually slow to develop (Germanier, 1984).

TB can spread through the air from one person to another. TB infection means that tubercle bacilli are viable within the body but are kept from multiplying and causing disease by the immune system of the host. People who have TB infection but not TB disease are not infectious, in other words, they cannot spread the infection to other people. Not all infected individuals develop tuberculosis. Disease due to tuberculosis in populations is influenced by three distinct risks: the risk of an individual in the community being infected with tubercle bacilli in a given time period, the risk of disease following shortly after such infection, and the risk of disease occurring long after the original infection owing to the reactivation of latent bacilli (Bloom, 1994). The risk of progression to TB disease is determined by the host immunity against the invading organisms. Certain conditions increase the risk of progression such as diabetes with TB infection are three times more likely to progress to the disease, and people with HIV infection are over 100 times more likely to do so.

The possibility that a person with no risk factor will develop TB is approximately 10%, and is the highest in the first two years after infection. The first manifestation of TB is called primary tuberculosis, being with a lesion known as Ghon's focus in the mid-zone of the lung. Asymptomatic cases may found on routine examination. Symptoms may include cough, fever, decreased appetite and, in infant,

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failure to thrive. Immediate complications include bronchopneumonia, pleural effusion and disseminated disease. Intermediate complications include the various forms of nonpulmonary disease such as bone and meningeal involvement. Late complications include bronchiectasis and postprimary tuberculosis. Tuberculin conversion, as indicated by a positive tuberculin skin test, usually occurs 3-8 weeks after infection. Response to standard treatment is usually good. The infection often lies dormant for many years, erupting into postprimary disease in old age (Davies, 2001).

Postprimary or secondary tuberculosis is the recurrence of TB in later life. There are two routes to a repeat episode of TB: either by inhalation of additional *M. tuberculosis* organisms or by reactivation of a dormant primary lesion. Tuberculosis due to reactivation of latent bacilli is presumed to result from a failure in immune surveillance. Like primary disease, postprimary disease may be asymptomatic. Additionally, in reinfection TB, a hypersensitivity reaction is the characteristic response, accompanied by tissue necrosis and caseation. In an attempt to seal off the necrosis site, lymphocytes and other cells converge upon the site and direct formation of a wall of fibrous tissue. Most often, caseated granulomas heal over time, shrinking as they become fibrotic and then calcified. However, if healing is impaired, the growing lesion may erode adjacent bronchi, resulting in the formation of cavities. *M. tuberculosis* organisms multiply freely in these cavities, leading to a large number of bacilli. An open cavited lesion can leak infectious material directly into the bronchus, resulting in the continuous discharge of bacilli into the sputum. Leaked *M. tuberculosis* bacilli can also be inhaled into other portions of the host's lungs, resulting in tuberculous bronchopneumonia. If the growing granulomatous lesion erodes the wall of the vein, organisms can spread in the circulating blood, resulting in miliary disease.

TB bacilli can persist for decades in a dormant state inside a granuloma. Reactivation of these latent organisms can also lead to postprimary disease, despite in person who sucessfully fought their initial battle against TB. The mechanisms which govern dormancy and reactivation, within either the organism or the host, are not yet understood (Fenton and Vermeulen, 1996).

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Death due to TB largely depends on the site and severity of the disease and timely diagnosis for its treatment. Approximately 30-40% of untreated sputum smear positive cases die within one year, and 50-70% within 5-7 years. The overall mortality from tuberculosis is approximately 8%, being over 30% in the elderly but less than 1% in the young (Davies, 2001).

B. History of tuberculosis

From the time immemorial tuberculosis has ranked amongst the most feared and dreaded of the numerous diseases that afflict mankind. The evangelist John Bunyan dubbed tuberculosis 'the Captain of all of these man of Death', and in Indian it was known as the King of Diseases (Grange, 1996). There are records indicating that pulmonary tuberculosis was recognized in Europe in pre-Christian times, and there was archeological evidence of tuberculosis lesions in mummified remains taken from early Egyptian tombs. Although tuberculosis was known to occur in Europe throughout the Middle Ages, such infections were thought to be relatively rare until the Industrial Revolution. The explosive increase in the incidence of tuberculosis seen at this time was probably due to the extensive urbanization, overcrowding, poor nutrition, widespread drunkenness, abysmal working conditions, ignorance, and superstition existing in Europe at that time (Germanier, 1984).

Tuberculosis may occurred as an endemic disease in animals long before it affected humans. *Mycobacterium bovis* was the most likely infecting organism, and the first human infections may have been with *M. bovis*. Since *M. tuberculosis* infects all primate species, it is also possible that this species existed in subhuman primates before it became established in humans. The epidemic slowly spread worldwide as a result of infected Europeans traveling to and colonizing distant sites. In the 1700s and early 1800s, tuberculosis prevalence peaked in Western Europe and the United States and was undoubtedly the largest cause of death, and 100 to 200 years later, it had spread in full force to Eastern Europe, Asia, Africa, and South America. Within a particular populatiopn in a defined geographic area, the tuberculosis epidemic reached its peak within 50 to 75 years after its beginning and then slowly declined, possibly as the more resistant host reservoirs reproduced (Bloom, 1994).

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C. Discovery of tubercle bacilli

The turning point in the history of tuberculosis occurred at the meeting of the Berlin Physiological Society on 24 March 1882, when Robert Koch described the isolation of the causative organism of tuberculosis. Koch's discovery heralded the era of hope, and serious research soon took off in three main directions: the isolation and culture of the bacillus for diagnostic purposes, the search for an effective cure, and the development of the vaccine. To carry out these directions, Koch developed staining techniques and was the first to employ culture on solid media. Very shortly afterwards, Paul Ehrlich discovered the 'acid-fastness' of the tubercle bacillus and introduced a staining technique which, with minor modifications by Ziehl and Neelsen whose names the method now bears, is still widely uesd today. Originally, tubercle bacilli were grown on heat-coagulated serum, then glycerol-beef broth, and egg-based media, which introduced by Dorest in 1902 and were modified by Lowenstein in 1930. Method for decontaminating clinical specimens were introduced by Petroff and others around 1915 (Grange, 1996). Koch's criteria for proof that the organism he discovered caused tuberculosis have been adopted and have become known as Koch's postulates. Koch went on to show that the tuberculous animals produces an accelerated inflammatory response at a secondary intradermal inoculation site, the so-called Koch phenomenon. The general principles of vaccination were well-established by Pasteur, and many workers attempted to attenuate the tubercle bacillus for use as a vaccine. A vaccine was eventually produced by Calmette and Guerin after passaging a bovine tubercle bacillus 230 times on potato sliced soaked in bile and glycerol over a period of 13 years. This vaccine, Bacillus Calmette-Guerin (BCG), was first used in 1921. Nowadays the efficacy of this vaccine remains unknown, but it currently use to prevent tuberculosis worldwide (Bloom, 1994).

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D. Transmission of *Mycobacterium tuberculosis*

The transmission of the disease occurs by spread through the air from person to person. The concept that when a person with tuberculosis of lung or throat coughs, spits, sneezes, talks or even sings, the bacteria are sprayed out into the air as infectious droplets. These droplets dry up rapidly and the smallest of them remain suspended in the air for several hours. A 10-μm droplet nucleus may carry perhaps 3-

10 tubercle bacilli. People nearby could easily breathe in TB bacilli and become infected. When a person inhale these bacteria, the organisms could settle in the lungs and begin to multiply. From the lung they could journey through the circulatory system to various part of the body. TB bacilli can remain viable in wet sputum for months and in dry sputum for weeks (Narain, 2002). Additionally, dust-associated particle may also carry *M. tuberculosis*. These particles are larger than droplets, but they can be transiently resuspended by air convection and may serve as a reservoir for infectious bacilli.

Most TB infections are acquired by continued exposure rather than casual contact, because respiratory defence mechanisms can kill small numbers of mycobacteria. However, infection can occur by ingestion of tubercle bacilli but is about 10,000-fold less effective than inhalation of droplets in transmitting tuberculosis, probably because tubercle bacilli are very sensitive to gastric acid. From the studied of Riley (1959), the clearest demonstration of the importance of the droplet nuclei for transmission. The finding that when air from the ventilating system of a hospital ward for tuberculosis patients was ducted through an exposure chamber that housed guinea pigs, 71 of 156 animals became infected with tuberculosis. Interestingly, in the same experiments, UV irradiation on the air from the same ward prevented transmission to guinea pigs (Bloom, 1994).

E. Treatment and preventation of tuberculosis

The aims of treatment are to cure patients, prevent deaths due to active tuberculosis or its complications, prevent relapses of TB, and decrease the transmission of disease. Tuberculosis is always treated with a combination of drugs to prevent drug resistance emerging. The current recommendation is that four drugs as follows isoniazid, rifampicin, pyrazinamide, and ethambutol should be given initially. These drugs should be continued for at least two months. A furthur four months of isoniazid and rifampicin are required to prevent relapse in the fully drug-sensitive patient (Davies, 2001).

A comprehensive strategy promoted by the World Health Organization, DOTS is the acronym for Directly Observed Treatment, Short-course. At the core of the strategy of DOTS, TB patients must take their drugs under the direct observation of a

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health worker or volunteer. DOTS is a form of patient support, the daily interaction helps to remind and motivate the patients to take their medication. These DOTS providers ensure that the patients continue the regimen, because non-compliance with treatment is the most important reason for the development of drug resistance. The risk of developing drug resistance is higher during the early stages of anti-TB drug treatment when there are more bacilli and therefore, DOTS is all the more essential in the initial phase. However, the effective treatment of TB is critical to the well being of the patient, their family and community. Treatments benefits the patient by preventing disability and death, at the same time it arrests the chain of transmission to the community (Narain, 2002).

The disease preventation, in principle, there are two approaches: preventive therapy and vaccination. Preventive therapy, giving isoniazid alone or a combination of drugs, to prevent tuberculosis infection or to prevent infected individuals developing disease, is widely used. The vaccination agent against tuberculosis is BCG, an attenuated form of *M.bovis*. In spite of being the most widely used vaccine worldwide, controlled trials have shown a wide variation in efficacy from 80% to zero. Where efficacy has been shown it only lasts for 15 years. No studies have shown efficacy for second or subsequent vaccinations. Because of the decline of tuberculosis in the white population, discontinuation of routine vaccination has been suggested. Nevertheless the current recommendation is that routine BCG should be continued because of the globally resurgence of tuberculosis (Davies, 2001).

F. Drug resistant tuberculosis

Drug-resistant tuberculosis is tuberculosis in which resistance to one or more anti-tuberculosis drugs. Drug-resistant tuberculosis is mostly a man-made phenomenon. The possible causes of the emergence of drug-resistant strains of tubercle bacilli include the widespread prescription of insufficient anti-TB drug regimens, failure to ensure a regular and uninterrupted supply of good quality drugs and poor case management wherein there is failure to ensure that the patient takes every dose of medicine under the direct observation of treatment. Resistance to an antibiotic is defined as growth of a bacterial culture even in the presence of a particular concentration of the drug in the culture medium.

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Multi-drug resistant tuberculosis (MDR-TB) is the laboratory documented resistance of TB bacilli to isoniazid and rifampicin, the two most active anti-tuberculosis drugs. MDR-TB can spread very rapidly, particular those in which immuno-compromised persons (Narain, 2002).

Drug-resistant tuberculosis is divided into two main types : primary resistance and acquired resistance. Primary resistance is defined as the presence of drug resistance to at least one anti-TB drug in a TB patient who has never received prior treatment. Acquired resistance is defined as resistance to at least one anti-TB drug that arises during or after the course of treatment, usually as a result of nonadherence to the recommended regimen or of faulty prescribing. A high level of this type of resistance is a mark of a poorly functioning TB control program.

In many industrialized countries, the incidence of both primary and acquired resistance was reduced by improving application of the same regimen used previously. In the other hand, in many developing countries, particularly in Asia, the incidence of acquired resistance remains high and the incidence of primary resistance is higher than that in industrialized countries, because national TB control programs in the developing countries have not been able to achieve a high cure rate over a very long period. The serious situation may quickly worsen as the HIV epidemic spreads, which can produce increased levels of both resistance types (Bloom, 1994).

G. Trend of tuberculosis

The global tuberculosis burden increasing for several reasons : poverty and widening gap between the rich and the poor, neglect of tuberculosis control, increasing world population and changing age structure, and the impact of HIV pandemic.

In most industrialized countries , HIV infection is expected to exert only a minor impact, as the age groups at particular risk of HIV infection are rapidly replaced by cohorts with virtually no tuberculosis infection. Therefore, the tuberculosis morbidity in these countries mostly depended on the migration trends and origin of immigrants. Although the absolute number of cases resulting from this type of transmission is relatively small. Hence, the trend of tuberculosis in these countries are likely to decline.

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In contrast, in developing countries show the bulk of the global TB burden. Nearly 95% of the estimated tuberculosis cases and 98% of estimated deaths occur in these countries. The HIV epidemic has caused marked increased in tuberculosis notifications in populations where there is overlap between those infected with HIV and those infected with tubercle bacilli. It is estimated that two-thirds were dually infected with HIV and TB, most of them in sub-Saharan Africa, followed by South East Asia. In Asia, rapidly increasing HIV seroprevalence levels among tuberculosis have been reported in northern Thailand and in certain areas in India. The situation remains precarious even for countries that are not seriously affected by HIV, but show a high prevalence of tuberculosis infection in the younger age groups. The course of the epidemic in such countries will depend on the success in implementing control strategies as swiftly as possible, with the overall aim of the reducing the risk of TB infection in the community (Narain, 2002).

H. *Mycobacterium tuberculosis*

Mycobacterium tuberculosis, an etiologic agent of tuberculosis, is slender rod, about 4 μm long and 0.5 μm in diameter. It is non-motile, does not produce capsule and spores. It is difficult to stain, but once stained with carbolfuchsin or fluochrome auramine. It resists to decolorization by mineral acids and alcohol, therefore It is known as acid-fast bacilli (AFB). The morphology and staining property of stained bacilli is not constant at all stages of the growth cycle hence pleomorphism is occasionally seen. Culture techniques are more sensitive than staining methods and allow drug susceptibility test to be made. The Lowenstein-Jensen culturing method is the most widely used.

M. tuberculosis is a strict aerobe and grows very slowly, deviding every 18-24 hours under optimum conditions. On culture media, *M. tuberculosis* colonies are rough, dry, hard-textured and ivory coloured and appear only after a minimum of two weeks incubation at 37°C. Culture in liquid media permits more rapid detection of growing organisms. The radioactive method of culture is even more rapid. The bacteria are incubated in liquid culture containing carbon 14, the radioactive isomer of carbon, the emission of radioactive CO_2 as a result of bacterial metabolism, which can be detected by a Gieger counter, indicates the presence of live bacteria. TB bacilli

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cannot utilize proteins, but the proteins protect the bacilli from the toxic substances in the medium. *M. tuberculosis* can utilize a wide range of compounds. The preferred carbon sources are glycerol, pyruvate and glucose. The preferred nitrogen sources is asparagine, but glutamate and aspartic acid are effective substitutes. Tubercle bacilli also require inorganic elements such as potassium, magnesium, sulphur, phosphorus, zinc and manganese, and trace elements such as iron, which has been reported that it is essential for *M. tuberculosis* growth in macrophages (De Voss et al., 2000).

The cell wall of the tubercle bacilli is one of the most complex of any bacterium characterized by a very high lipid content. Approximately lipid content for about 60 percent of the cell wall weight and they consist of a wide range of compounds, some being similar to those found in other organisms and others being unique to the mycobacteria. Like most bacteria, the cell wall of *M. tuberculosis* is essentially composed of peptidoglycan. This, as in other bacteria, consists of long polysaccharide chains cross-linked by short peptide chains, thereby forming a net-like macromolecule that gives the cell its shape and rigidity. Moreover, mycobacterial peptidoglycan is notoriously resistant to lysozyme, and, although the evidence is not conclusive, it appear that the N-glycolyl group on the muramic acid residue in the peptidoglycan may protect the organism from degradation. External to the peptidoglycan is a layer of arabinogalactan, a branched polysaccharide composed of arabinose and galactose. The terminal arabinose unit on the side chains are covalently linked to a group of long chain fatty acids termed mycolic acid. These form a dense palisade which gives the cell wall its thickness and is probably responsible for acid fastness. The mechanism for the synthesis of mycolic acid is the target for the antituberculosis drug isoniazid. Lipoarabinomannan (LAM) is a branched polysaccharide, composed of arabinose and mannan, link to a phospholipid of the cell membrane. This molecule appears to stretch from the membrane up to the surface. There are two types of LAM, AraLAM, which has arabinose on the branches, and the other is ManLAM which the branches mostly capped with mannose. LAM is a dominant mycobacterial antigen and, in the case of pathogenic species, it has a number of important effects on the immune response of the host. There have been reported that LAM is a potent inhibitor of IFN-γ-mediated activation of mouse macrophage in vitro (Sibley et al., 1988). It was found that LAM isolated from a

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virulent strain and from an avirulent strain of *M. tuberculosis* have been shown to differ markedly in terms of the structure of their reducing termini, also differ markedly in the capacity to induce the secretion of tumor necrosis factor from murine macrophages. The result from their study demonstrated that LAM from the avirulent strain was 100-fold more potent at inducing tumor necrosis factor secretion than LAM from the virulent strain, therefore leading them to hypothesize that the structure of LAM from a given isolate may directly influence its ability to elicit, or avoid cytokine-mediated mechanism of host resistance (Chatterjee et al., 1992). Moreover, it has been reported that LAM can inhibit various IFN-γ-induced functions including macrophage microbicidal and tumoricidal activity (Sibley et al., 1988) and it can act as a scavenger to scavenge the potentially cytotoxic oxygen free radicals and inhibit protein kinase C activity (Chan et al., 1991). The outer layer is composed of a heterogenous group of peptidoglycolipids or phenolic glycolipids termed mycosides. The mycobacterial cell wall also contains a range of other lipids as well as various proteins and polysaccharides (Grange, 1996).

The genome of *M. tuberculosis* is a singular chromosome comprised of 4,411,532 base pairs (bp) and contains around 4,000 genes (3,924 ORF) with high GC content of 65.6%. Consistent with the high GC content of the genome, GTG initiation codon (35%) are used more frequently than in *B.subtilis* (9%) and *E.coli* (14%), although ATG (61%) is the most common translation start. There are about 250 distinct enzymes involved in fatty acid metabolism in *M. tuberculosis* compared with only 50 in *E.coli* which related to its cell wall components which composed with high lipid content (Cole et al., 1998).

I. Laboratory diagnosis of *Mycobacterium tuberculosis*

Specimen collection

Specimen must be collected in clean, steriled containers and held under conditions that inhibition the growth of contaminants, since most specimens will contain bacteria other than mycobacteria. Sputum is the specimen most often collected and processed, but other specimens such as fluids collected by gastric or bronchial lavage, urine, tissue from any organ, spinal fluid and blood may be collected. All specimens from nonsterile sites are similar in that they must be

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decontaminated before being cultured on the medium. A series of three to six early-morning sputum specimens should be collected on successive days before the start of chemotherapy and sent to the laboratory without delay (Bloom, 1994).

Staining and smear examination

As the isolation of *M. tuberculosis* and most other pathogenic mycobacteria by standard cultural methods takes several weeks, the use of microscopy to reach a preliminary diagnosis is of great importance (Grange, 1996). Smear may be made directly from untreated specimen or from concentrated specimen. The acid-fast staining procedure depends on the ability of mycobacteria and some other microorganisms to retain dye even when treated with mineral acid or acid-alcohol solution. Two procedures for acid-fast staining are widely used, those using the Ziehl-Neelsen, which mycobacteria will appear as red-stained rods or coccobacilli on a blue background. Another one is the auramine O fluorescence acid fast stains, which mycobacteria will appear as yellow fluorescing rods on a dark background. Smear must be examined carefully, and a preparation cannot be considered negative until it was examined equivalent of 300 oil immersion fields. Those specimens that contain only three or fewer acid fast bacilli (AFB) in 300 oil immersion fields are considered doubtful and should be retested. A sputum smear positive for AFB may represent either *M. tuberculosis* or some nontuberculous mycobacterium. The result from the staining and smear examination is the first report issued following receipt of a specimen, and it can be a useful instruction regarding the adequacy of disease therapy (Bloom, 1994).

Isolation of *Mycobacterium tuberculosis*

Nowadays Lowenstein Jensen (LJ) medium, the most popular media contains egg, asparagine, glycerol and some mineral salts, is widely used to the isolation of *M. tuberculosis*. Egg-based media usually contain a dye such as malachite green which, in addition to being inhibitory to certain contaminating bacteria, give a better background color against which colonies of mycobacteria are more clearly seen. There are a number of clear broth or agar-based solid media but these are used more for drug susceptibility testing and research work than for primary isolation of strains

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from clinical specimens. Mycobacteria are aerobic but their growth is enhanced by an atmosphere of 5-10 % CO_2 in air. Most mycobacteria grow at 35-37°C. The slope of growth should be examined weekly for at least 8 weeks.

Because of the slow rate of growth of tubercle bacilli and most other pathogenic mycobacteria, the rapid methods were developed for detecting mycobacteria in specimens by their growth or metabolism. The only such rapid method in routine clinical use is radiometry. This technique based on the release of radioactive CO_2 from a labelled precursor by bacterial metabolic products. The released gas is detected by periodic sampling of the air space over the medium, this is done automatically in a commercially available instrument (Bactec 460/TB, Becton Dickinson).

The three techniques that are increasingly being use in diagnostic laboratories are polymerase chain reaction (PCR) to detect mycobacterial DNA in clinical specimens, nucleic probes to identify culture, and restriction fragment length polymorphism (RFLP) analysis (DNA fingerprinting) to compare strains for epidemiological purposes. Although such molecular techniques are not widely available to isolate or identify mycobacteria in routine laboratory (Grange, 1996).

J. Apoptosis

The phenomenon of physiological cell death has been discovered independently several times over the past 150 years. In 1972, Kerr et al (1972) published a seminal describing the novel physiological process of apoptosis (derived from the Greek word for "falling off"). The apoptosis program is a conserve feature of all eukaryotic cells from worms to mammals and constitutes a series of cellular biochemical events through which cells actively orchestrate their own demise. Apoptosis is essential for development of metazoans and is also crucial for the maintainance of cellular homeostasis in mammals. This genetic pathway of cell demise responds to normal and pathological stimuli. Additionally, apoptosis probably participates in the development of all cell lineages. It also plays essential role in the immune system. Aberations in genetic level of proteins which implicated in this process leads to the initiation of conditions such as autoimmunity, immunodeficiency and cancer. The genetic and molecular mechanismsm of apoptosis were first characterized in the late

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1980s in studies of the nematode worm *Caenorhabditis elegans*. Studies of the worm revealed that apoptosis consists of four sequential steps: (1) commitment to death by extracellular or intracellular triggers, (2) cell execution by activation of intracellular proteases, (3) engulfment of the cell corpse by other cells, and (4) degradation of the cell corpse within the lysosomal of phagocytic cells. These stages and the genes that govern them are remarkably conserved throughout animal evolution, from worm to human (Strasser et al., 2000; Gavrilescu and Denkers, 2003).

There are two principal patterns of cell death, apoptosis and necrosis. The cells of multicellular organisms generally die in 1 of 2 patterns as mention above depending on the context and cause of death. Apoptosis is an integral part of development and of homeostasis in adult tissue but necrosis is a passive, catabolic, pathological cell death process which generally occur in response to external toxic factors such as inflammation, ischaemic or toxic injury (Wu et al., 2001). The morphological features of apoptosis and necrosis found in different forms. Cells undergo apoptosis exhibit the condensation of cytoplasmic and nuclei, which leads to marked cell shrinkage. Other hallmarks include internucleosomal clevage of chromatin, blebbing of the plasma membrane and the exposure of cell surface molecules that signal phagocytosis of dying cell. A key consequence is that the cellular membrane tends to remain intact throughout apoptosis, preventing the leakage of cellular contents, which in turn ensures a relatively noninflammatory process. In contrast, necrotic cells swell and lyse resulting in releasing their cytoplasmic and nuclear contents into the intracellular milieu to result in occuring inflammation.

There are four major functional groups of molecules involved in triggering and affecting the apoptosis process. There are the caspases, the adaptor proteins, members of the tumor necrosis factor receptor (TNF-R) superfamily, and members of the Bcl-2 family of proteins (Hetts, 1998 ; Opferman and Korsmeyer, 2003).

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Caspases

Caspases, a group of cysteine aspartate specific protease, have a crucial role in programmed cell death in a variety of species. These enzymes recognize tetrapeptide motif and cleave their substrate on the carboxyl site of an aspartate residue. Caspases are synthesized as zymogens, which have very low intrinsic enzymatic activity. The

active form of enzymes are heterotetramers composed of two identical large subunits (~20kDa) plus two identical small subunits (~10kDa). These subunits can be produced by caspase-mediated clevage. These enzymes can be boardly divided into two groups namely initiator caspases, which main function is to activate downstream caspases , and the other is executioner caspases, which are responsible for dismantling cellular proteins. During apoptosis, iCAD, an inhibitor of the caspase-activated DNase (CAD), is cleaved by caspases, and this leads to release of the active endonuclease, which produces the characteristic internucleosomal clevage (Strasser et al., 2000).

Adaptor proteins

The adaptor proteins, which control the activation of initiator caspases, are the links between the three classes molecules, the cell death effectors, caspases, and the cell death regulators, death receptors, and Bcl-2 family members. These links resulting in physical association between these molecules. The adaptor proteins act as the bridges between caspases and upstream regulation of apoptosis such as association between adaptor proteins and caspases or TNF-R family members are mediated by homotypic interaction for instance between the death domain (DD), the death effector domain (DED), and the caspase recruitment domain (CARD). After cross-linking of these molecules, the homotypic interaction between its DD and that of the adaptor allow caspase aggregation and activation resulting in activation of downstream caspases and activation of apoptosis cascade, respectively (Strasser et al., 2000).

The tumor necrosis factor receptor (TNF-R) family

The members of the TNF-R family have pleiotropic action. These receptors can trigger proliferation, differentiation, survival and death depending on the cell types and the other signals that the cells are received (Strasser, 2000). Additionally, these receptors can be subdivided into two major groups on the basis of their cytosolic signaling domains : those that have a death domain (DD) and activate apoptosis, and those that have a short peptide-recognition motif for the TNFR-associated factor (TRAF) family of adaptor. The DD receptor such as Fas, TNF-related apoptosis-inducing ligand (TRAIL)-R1, TRAIL-2 and TNFR1, initiate apoptosis signaling

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through the recruitment of DD-containing adaptor proteins (e.g. the Fas-associated DD-containing protein [FADD]) and subsequent activation of the caspase family of protease (Benedict et al., 2003).

The Bcl-2 family

Bcl-2 is a human proto-oncogene located on chromosome 18. It was first found in follicular(B) lymphoma. Its product is an integral membrane protein called Bcl-2 located in the membranes of the endoplasmic reticulum (ER), nuclear envelope, and in the outer membranes of the mitochondria. Increased expression of Bcl-2 gene led to reduced cell death. Several members are now found with two contrasting effects, pro-survival (anti-apoptosis) and the other pro-apoptosis. There are three structural classes, Bcl-2 subfamily (pro-survival), Bax subfamily (pro-apoptosis), and BH3-only subfamily (pro-apoptosis). BH-3 only proteins operate as upstream sentinels, selectively sensing both intrinsic and extrinsic death stimuli, and then communicate this information to the pro-apoptotic members. This process is antagonized by anti-apoptotic members of the Bcl-2 family (Opferman and Korsmeyer, 2003).

K. *Mycobacterium tuberculosis* and its host cell, the macrophages

Macrophages

In the bone marrow of mammalian organisms, macrophages are derived from myeloid progenitor cells and granulocyte-monocyte colony-forming units, which ultimately develop into monocytes that enter the peripheral bloodstream. Monocytes have kidney-shaped nuclei and assume a large cell body during further differentiation and activation. Throughout life, some monocytes adhere to and migrate through the endothelium of the capillaries into all organs, where they differentiate into resident tissue macrophages or dendritic cells.

One of the key functions of macrophages was first seen by the German zoologist Ernst Haeckel in 1868 is observed in protozoa such as free-living amoebae and *Radiolaria*, which are characterized by the ability to engulf live and dead matter from their environment. In 1882, the Russian biologist Elie Metchnikoff recognized the presence of highly mobile mononuclear phagocytes in invertebrates. Based on this observation demonstrated that these cells take up and digest bacteria and organic

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matter, a process that later termed phagocytosis. From this observation, Metchnikoff postulated that in complex higher organisms phagocytes patrol the various organs and epithelial surface in order to remove nonself material, ranging from aged or malignant cells to microbial invaders. This revolutionary theory turned out to be true (Bogdan, 2001).

Macrophages are involved in all stages of the immune response. They can act as a first-line nonspecific defence against invading microorganisms before an antigen-specific T cell-mediated response can be mounted. They then help to initiate a T-cell response by processing and presenting antigen. They also responsible for the secretion of cytokines, chemokines and other soluble mediators. Finally, they can act as effector cells with tumoricidal, microbicidal and inflammatory activity, all of which are under T-cell regulation. The first, nonspecific, defence function depends largely on their capacity to take up by a procedure known as phagocytosis particulate material such as bacteria and parasites, antigen-antibody complexes, cellular debris senescent erythrocytes and apoptotic cells. Some of these functions are constitutively expressed. However, in most cases exogenous signals delivered by cytokines, interaction with T cells or certain extracellular matrix components are required to make the macrophage fully competent to kill tumor cells, or destruction of intracellular pathogens or of antigen presentation. This process is most commonly called macrophage activation. Many of products released by activated macrophages such as reactive oxygen and reactive nitrogen intermediates, lysosomal acid hydrolases, collagenases and plasminogen activator can damage the tissue and cause harm to the host organism. During an antimicrobial immune response the activity of macrophages therefore needs to be controlled and eventually downregulated in order to avoid ongoing inflammation after the microbial pathogens have been killed. This process has been termed macrophage deactivation and is mediated both by cross-linking of inhibitory receptors, by anti-inflammatory cytokines and by nonprotienaceous compounds (Peracchia et al., 2001).

Entry and survival of *M. tuberculosis* in macrophages

A crucial task of macrophages is to recognize and endocytose a large number of genetically diverse infectious pathogens and to discriminate these from noninfectious,

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innocuous, intact self structures. In addition, macrophages are also important for the clearance of senescent and apoptotic cells in the host organism. The resurgence of concern about tuberculosis has resulted in the discovery that *M. tuberculosis* has developed numerous mechanisms for entering human macrophages. Binding and phagocytosis of *M. tuberculosis* to macrophages can be mediated by different kinds of receptors including complement receptors (CR), mannose receptors, surfactant receptors, scavenger receptors, and GPI-anchored receptors (such as CD14).

Complement receptors

Phagocyte complement receptors occur in two distinct structural forms. Complement receptor type 1 (CR1) is a monomeric transmembrane protein that binds C3b and C4b but not C3bi. CR1 possesses complement regulatory activity and can mediated phagocytosis of bound particles. CR3 and CR4 are heterodimeric proteins of the integrin superfamily. CR3 and CR4 bind C3bi, and CR3 also contains a glycan binding site (Ernst,1998). Different from other bacteria such as *Staphylococus aureus* and *Listeria monocytogenes* or other intramacrophage pathogens such as *Leishmania mexicana,* pathogenic mycobacteria, including *M. tuberculosis*, have developed an additional mechanism for aquiring opsonic C3 peptides. Pathogenic mycobacteria surpassing recruit the complement fragment C2a to form C3 convertase and generate opsonically active C3b in the absence of early activation complements of alternative and classical pathway (Schorey et al., 1997). *M. tuberculosis* can bind to CR3 at two disparate sites on the receptor. Opsonized *M. tuberculosis* binds CR3 at its C3bi binding domain, and nonopsonized *M. tuberculosis* uses its endogenous capsular polysaccharides to interact with β-glucan binding site near the C-terminus of CD11b (Cywes et al., 1996; 1997; Velasco-Velazquez et al., 2003).

Mannose receptors

Mannose receptors are expressed on mature macrophages but not on fresh monocytes. The macrophage mannose receptor is a monomeric transmembrane protein, with an extracellular domain containing eight carbohydrate-recognition domains characteristic of C-type (calcium-dependent) lectins (Taylor, 1993). Human monocyte-derived macrophages bind and internalize virulent *M. tuberculosis* via

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mannose receptor (Schlesinger, 1993). The expression of mannose receptor is downregulated by gamma interferon, therefore, their role in ingestion of *M. tuberculosis* early in infection or in individuals with compromised cellular immunity may be more important than in established granulomas (Schreiber et al., 1993).

Surfactant protein A and surfactant protein A receptors

Surfactant protein A (Sp-A) is a member of collectin family of proteins, which includes serum mannose binding protein (MBP) and complement component C1q. Sp-A like other collectins, possesses a collagen-like domain and has a domain resembling C-type lectins such as that in MBP. Binding and uptake of *M. tuberculosis* by macrophages are enhanced by Sp-A, however, the mechanisms of these phenomena have not been fully elucidated (Downing et al., 1995). From the studied of Gaynor (1995), suggested that Sp-A is not simply acting as an opsonin, binding to *M. tuberculosis* by the N-linked polysaccharides of Sp-A and to macrophages by the collagen-like domain, it may be modulating the activity of one or more receptors that are responsible for directly binding *M. tuberculosis* (Gaynor et al., 1995).

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