Chapter 3

The Existence of *Mycobacterium tuberculosis* in Microenvironment of Bone

Rahyussalim Ahmad Jabir, Andriansjah Rukmana, Ifran Saleh and Tri Kurniawati

Additional information is available at the end of the chapter

http://dx.doi.org/10.5772/intechopen.69394

Abstract

Mycobacterium tuberculosis is an obligate aerobe bacteria requiring oxygen in its metabolism. In normal condition, bones have pH of 6.9–7.4 and temperature of 37°C. With the composition mentioned, bones fall in the group of tissue with less rich oxygen (<35%) which theoretically means, *M. tuberculosis* is hard to grow in the bone environment. Bone microliving environment is formed by the cells constructing the bone itself and the active cells which periodically interact with the bone cells. Activation of these cells gives impact to the temperature, pH, gas concentration, and liquid concentration, and at the same time triggers calcium, phosphor, and other minerals to be deposited in the bone. In the process of new bone formation, the osteoblast cells produce matrix and release them to the microenvironment that needs a high concentration of calcium and phosphor. The survival of *M. tuberculosis* in the microenvironment of bone is reflected in interaction of the bacteria and the non-immune cells, the bacteria and the organic environment, and the bacteria and the inorganic environment. In addition, the immune system also threatens the survival of *M. tuberculosis*. The results of these interactions will affect the lives of bacteria and has an impact on the bone microenvironment.

Keywords: bone microenvironment, survival of *M. tuberculosis*, cell host interaction, organic bacteria interaction, inorganic bacteria interaction

1. Introduction

Mycobacterium tuberculosis is an obligate aerobe bacteria requiring oxygen in its metabolism processes. Because of this oxygen requirement, *M. tuberculo*sis manifests in the lung of mammals

that have very high volume of oxygen. The optimum growth condition for this bacterium is at 37°C, pH 6.4–7.0, and oxygen level of >95% [1, 2]. Bones are composed of matrix comprising 60–70% inorganic components, 5–8% water, and the rest is organic components. In normal condition, bones have pH of 6.9–7.4 and temperature of 37°C. With the composition mentioned, bones fall in the group of tissue with less rich oxygen (<35%) which theoretically means, *M. tuberculosis* will be hard to grow in the bone environment [3].

In reality, however, *M. tuberculosis* can live in and infect the bone. This is proven in the cases of tuberculosis spondylitis and osteomyelitis. How this happens, what are the mechanisms that exist, and what compounds and conditions in control to enable *M. tuberculosis* growth in the bone environment will be discussed in this chapter.

2. *M. tuberculosis* complex

2.1. Cellular structure of *M. tuberculosis*

M. tuberculosis is a type of Actinomycetales bacteria of the Mycobacteria family and *Mycobacterium* genus (**Figure 1**). Shaped as tiny thin rod-shaped tubercle bacilli, *M. tuberculosis* is straight or slightly curved with 2–4 μm long and 0.2–0.5 μm wide, depending on the environment condition. When observed under the light microscope, this bacterium is usually conjoined forming a chain, filament, or branched forming into X, Y, or V shape [4].

M. tuberculosis does not have any capsules, and the cell walls (**Figure 2**) comprising peptidoglycan and DAP (diaminopimelic acid), with lipid content of +60%, have metachromatic granules known as *Much* granules. The fat in the cell wall associated with

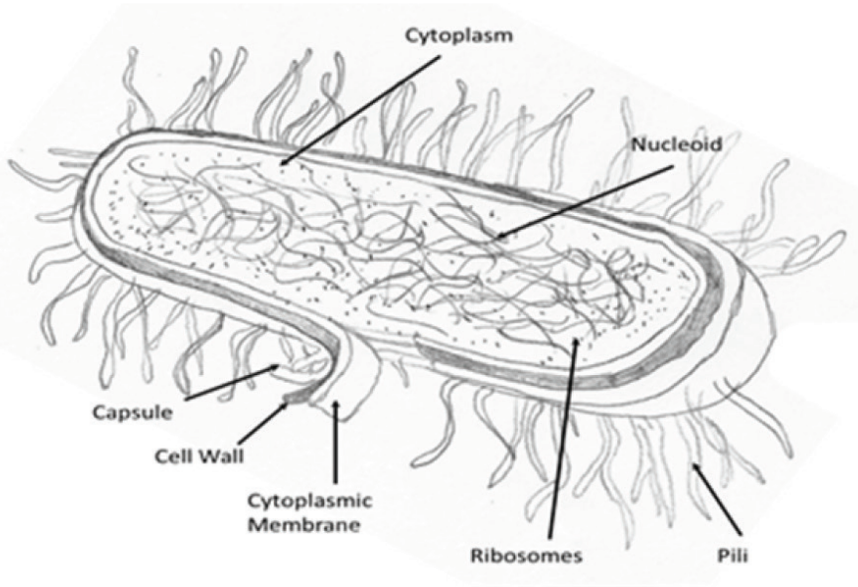

Figure 1. The structure of *M. tuberculosis* [4].

arabinogalactan and the peptidoglycan below forms a structure causing decrease in cell wall permeability that results in the reduction of antibiotic effectivity. Another molecule in the cell wall, lipoarabinomannan, is involved in the interaction between the host and the pathogen, making this bacterium survive in the macrophage [5].

Although *M. tuberculosis* does not produce spores, this bacterium is relatively heat resistant. The capability of adaptation in various microenvironment of *M. tuberculosis* is presented in **Table 1**.

Dormant is the effort of the bacteria to transform into the most stable form with a very low metabolism process and stop growing. This transformation is a response to the unsupportive environmental condition to grow normally. If one day the environmental condition becomes normal again and enable growing, this bacterium can revive and become active again [6].

2.2. Pathogenicity

2.2.1. Types

The known *M. tuberculosis* species which can infect human is classified into seven spoligotypes: The East African-Indian (EAI) strain and the Manu (India) strain, Beijing strain, Central Asian (CAS) strain, Ghana dan Harleem (H/T) strains, Latin America-Mediterranean (LAM) and X strains, *Mycobacterium africanum,* and Horn of Africa strains. This classification is according to the evolutionary demography of the bacteria [7, 8].

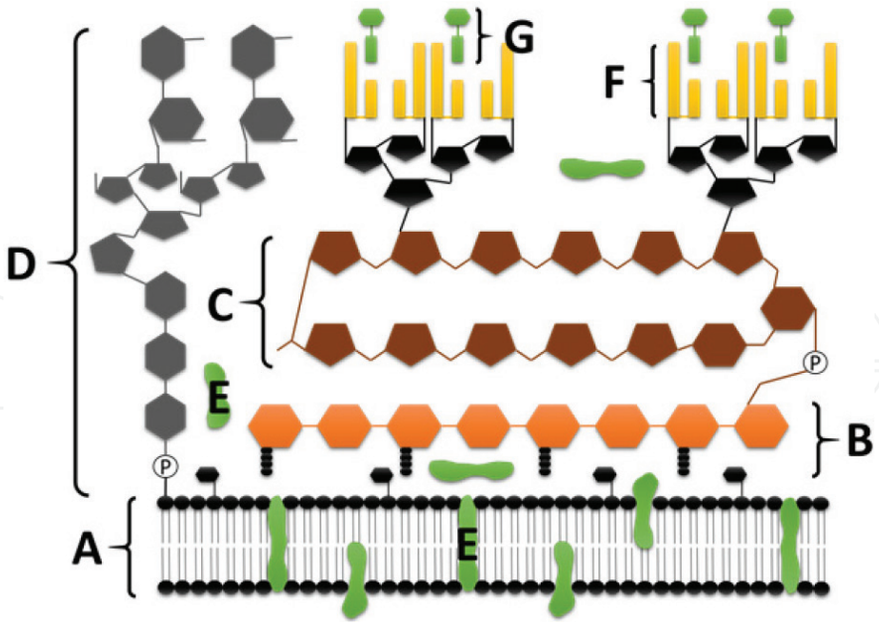

Figure 2. Structural and functional analysis and spatial organization of the "cell envelope" constituent of *M. tuberculosis* consisting of: plasma membrane (A), peptidoglycan (B), arabinogalactan (C), mannose covered with lipoarabinomannan (D), plasma membrane and the related cell-envelope-protein (E), mycolic acid (F), and glycolipid surface molecule associated with micolic acid (G) [5].

Environment	Description	Survival	Survival mechanism
Sunlight	Environment exposed to sunlight will result in low humidity and oxygen level. The dominant factor is when there is direct exposure with temperature reaching more than40°C and the period of the exposure	Survive in 2 hours	The cell wall thickened and produce liquid to protect from light exposure and high environment temperature
Dark and humid	Humid and dark condition will make the environment cool and rich in water. The oxygen level tends to be normal than the pH tends to be neutral	Survive according to the incubation period	Like in normal condition
Sputum	Sputum's temperature follows the temperature of the body, which is 36–37°C, pH of the sputum also follows the condition of other body fluid. The oxygen level inside the sputum is relatively high because the sputum lies inside the cavity of the lungs and the respiratory tract where oxygen flows	Survive in 20–30 hours	Like in normal condition
Storage cabinet	Storage cabinets have an advantage where the desired environmental temperature can be set according to the optimum condition favorable for the growth and proliferation of *M. tuberculosis*	Survive up to 2 years	Depends on the condition and the temperature set, normal or transformed into dormant
Mucosa of upper respiratory tract	The mucose of the respiratory tract has pH between 3.5 and 5.5, high oxygen level, and temperature close to body temperature	Ideal condition, reproduced and proliferated according to the period of incubation	Like in normal condition
Cavity of lung alveoli	The pH of the lung alveoli microenvironment is relatively higher compared to the pH of the upper respiratory tract, but still close to neutral pH for alveoli has the function to maintain the pH of the body. The oxygen level is relatively high and the temperature is warm because the activity of gas exchange and very active cell metabolism	Ideal condition, reproduced and proliferated according to the incubation period	Ideal condition
Macrophage's intracell	Ideal condition for the growth and development of *M. tuberculosis*. The intracell environment has a neutral pH with temperature of 36–37°C and oxygen level of >60%	Ideal condition, reproduced and proliferated according to the incubation period	Ideal condition
In tubercle	The environment inside the tubercle is also an ideal environment just like in the intracell, only this environment formed by the immune response trying to isolate the *M. tuberculosis*	Ideal condition, reproduced and proliferated according to the incubation period	Ideal condition

Environment	Description	Survival	Survival mechanism
Interstitial space of cancellous bone	Depends on the microenvironment, generally the oxygen level is low, the pH tends to be acid, and the temperature follows the body temperature	The condition is according to the normal incubation period	Normal or transformed into dormant
Interstitial space of cortical bone	Depends on the microenvironment, generally the oxygen level is lower compared to the interstitial space of the cancellous bone, the pH tends to be acidic, and the temperature follows the body temperature	The condition is according to the normal incubation period	Normal or transformed into dormant

Table 1. The survival of *M. tuberculosis* in various microenvironment.

2.2.2. Virulence factors and survival of M. tuberculosis

Full genome sequence of *M. tuberculosis* strain H37Rv was done successfully in 1998, but not all functions of the genes in the genome were known [1, 9]. From the discovery, it is known that *M. tuberculosis* do not have virulence factors like those discovered in other bacteria, such as toxin, capsule, or fimbriae, but some of the structures and physiological systems of *M. tuberculosis* itself contribute to the virulency. The virulence factors of *M. tuberculosis* among others are:

(a) *M. tuberculosis* can interfere with the toxic effect of reactive oxygen intermediate produced in the process of phagocytosis.

(b) *M. tuberculosis* has an antigen complex function to protect the bacteria from immune system and facilitate the formation of tubercle.

(c) Slow regeneration time of *M. tuberculosis* causes the immune system not recognizing this bacterium and eliminating it.

M. tuberculosis also have self-defense mechanism related to the virulence factors, one of them may be seen surviving extracellularly and intracellularly. Extracellularly, *M. tuberculosis* tries to survive by adapting to the environment through various mechanisms, such as decreasing metabolism, thickening the cell walls, reducing the surface area, and increasing the effectiveness of cell communication with the external environment. Intracellularly, when phagocyted by macrophage and finding a new environment different from the extracellular environment, the bacteria will feel threatened and tries to adapt by proliferating actively and inhibiting the fusion process of phagosome-lysosome so that it could not be digested [9].

In the effort to adapt to the environment and as a self-defense mechanism, bacteria could turn themselves into an inactive state, a condition where the bacteria do not give any respond to the environment they are in. The factors causing inactive bacteria to be infectious are: immune system and bacterial virulence. These two factors are highly related to one another in causing infection. For example, a weak immune system and a strong bacterial virulence will result in infection; this is also true in the condition where the immune system is weak and the bacterial virulence is also weak, the infection will still occur. However, it is not the case when the bacterial

virulence is strong and the immune system is also strong, because the infection will not occur in this condition. In MHC polymorphism, the host, genetically, has a condition most favorable to the bacteria to grow and develop.

Besides the immune system and bacterial virulence, the condition of the bone microenvironment, such as temperature, pH, oxygen level, and liquid, may also influence the existence of the bacteria as well as the interaction between the host and the bacteria. This phenomenon occurs because the living cells, indirectly, will influence the microenvironment from the metabolism products of the living cells. As an example, the debris of *M. tuberculosis* could influence the growth of the bone's active cells to form a favorable environment for bacterial growth. The bacteria will be active and dominant that the growth expanded and causing the bone's cells death. The death of bone cells will result in the formation of sequesters, which will then be deserted by the bacteria in order to find a new environment more favorable to maintain the bacterial existence and life (creeping phenomenon).

2.2.3. Immunology and defense mechanism

Response mechanism of the host to the intracellular pathogen bacterial infection depends highly on the location of infection. In immunology reaction or inflammatory reaction, a substance in the form of hormone and other cells functioned as intracell signal will be released by T lymphocytes, known as lymphokines. There are several lymphokines important to the process of *M. tuberculosis* infection, i.e., macrophage chemotactic factor, lymphocyte activation factor, and gamma interferon. In tuberculosis lesion, lymphokines from the T cells will cause macrophage accumulation and activation, and increasing number of TNF-alpha and TGF-beta lymphocytes will result in tissue damage [8].

In the first phase of *M. tuberculosis* infection, phagocytosis by the macrophages will occur as the result of bacterial activity in phagosomes. For 2–6 weeks, granuloma formation facilitated by CMI will happen, and the bacteria will then live and sit forever in the middle of the granuloma. The macrophage-bacteria interaction is initiated by the linkage between the bacterial cell wall and the macrophage at the time of phagosome-lysosome fusion. The inhibition of bacterial growth, even death, will further occur, inflammatory reaction and T cells antigen presentation will subsequently appear [8].

In the first stage of infection, right after the host exposed to the bacteria, detectable symptoms or immune response have not yet appeared. If the process of infection develops into the next stage, the signs of infection will then appear, for example, the skin tuberculin test and roentgen examination will give positive results. However, this process is not linear with the results of testing in the cell level and in the host organism level, where there will be shift between latent infection and the newly developed infection with the reactivation of previous infection [8, 10].

M. tuberculosis is an intracell microorganism that is needed in cellular immune response, which is the function of T-lymphocytes. In the thymus, T cells express surface antigens, CD4, CD5, and CD8, which in further development reside and mark the subset of T cells. The lymphocyte cells that act in the CMI reaction in tuberculosis infection are helper T lymphocyte cells (CD4), and suppressor T lymphocyte cells (CD8) are cells with specificities and functions tightly controlled by MHC (**Figure 3**). Based on the distribution in the tissues and molecule structure, MHC

antigens in human are divided into two main classes: class I antigen comprises HLA-A, HLA-B, and HLA-C, and class II antigen comprises HLA-D, HLA-DR, HLA-DQ, and HLA-DP [9].

M. tuberculosis is phagocyted by macrophage functioning as APC (**Figure 4**). This antigen is secreted by the bacteria together with MHC Class II and will react with CD4 on the T receptor and release IL-1, which further will replace CD4. This signal will give sign to lymphocytes to produce lymphokines, including gamma interferon, IL-2, BCGF, and chemotactic factor. Gamma interferon will activate macrophage to destroy the intracell *M. tuberculosis*. In this condition, the reaction between the somatic part of the bacterial antigen, which reacted with CD4 through the expression of MHC Class II with macrophage as APC, will occur. This active macrophage will cause some changes, such as increasing activity of hydrolase and increasing glucose metabolism [8].

Helper T cells composed of two subpopulations with different functions in producing cytokines (**Figure 5**). Th1 cells produce gamma interferon, IL-2, and lymphotoxin which are functioned to alter the macrophage's microbicide activity and strengthen DTH reaction. Th2 cells produce IL-4, IL-5, IL-6, and IL-10, which are functioned to assist the growth and differentiation of B cells and strengthen the humoral immune response. Th1 and Th2 cells will also produce IL-3, GMCSF, and TNF [11].

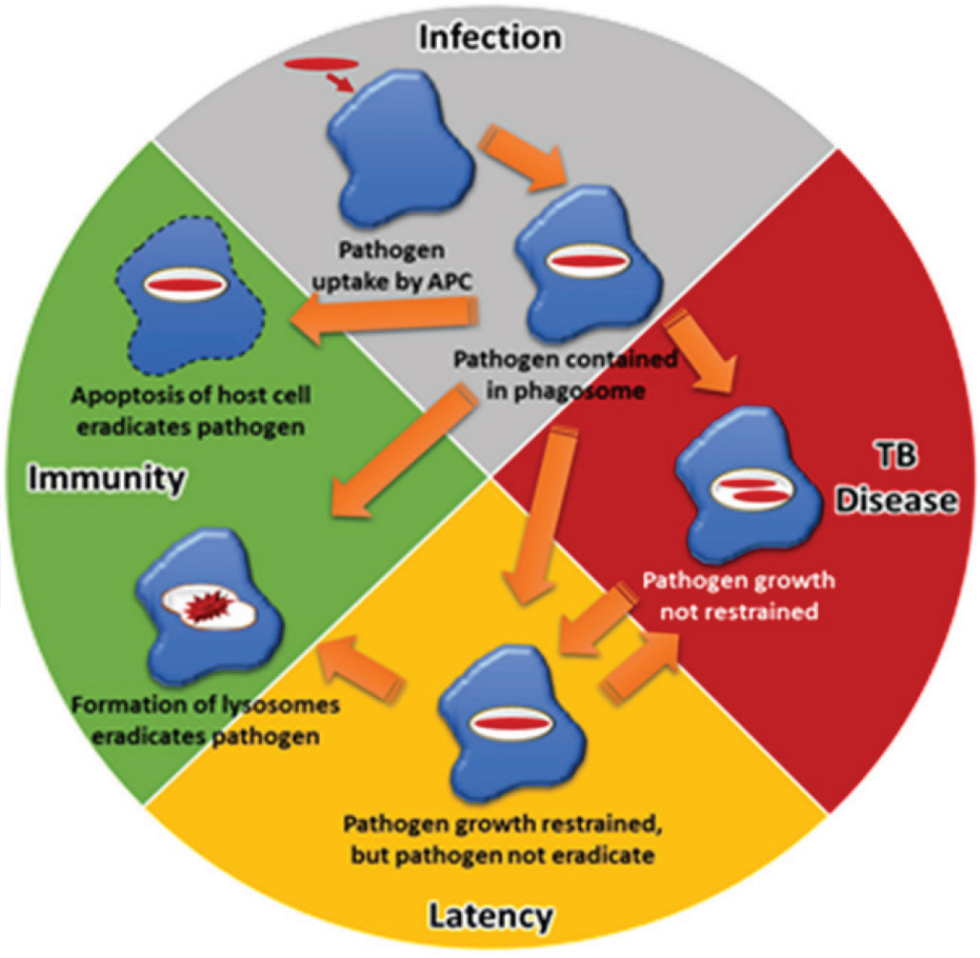

Figure 3. The relation between *M. tuberculosis* infection (new and latent) and immunity in infected host in the cell level (macrophage) [10].

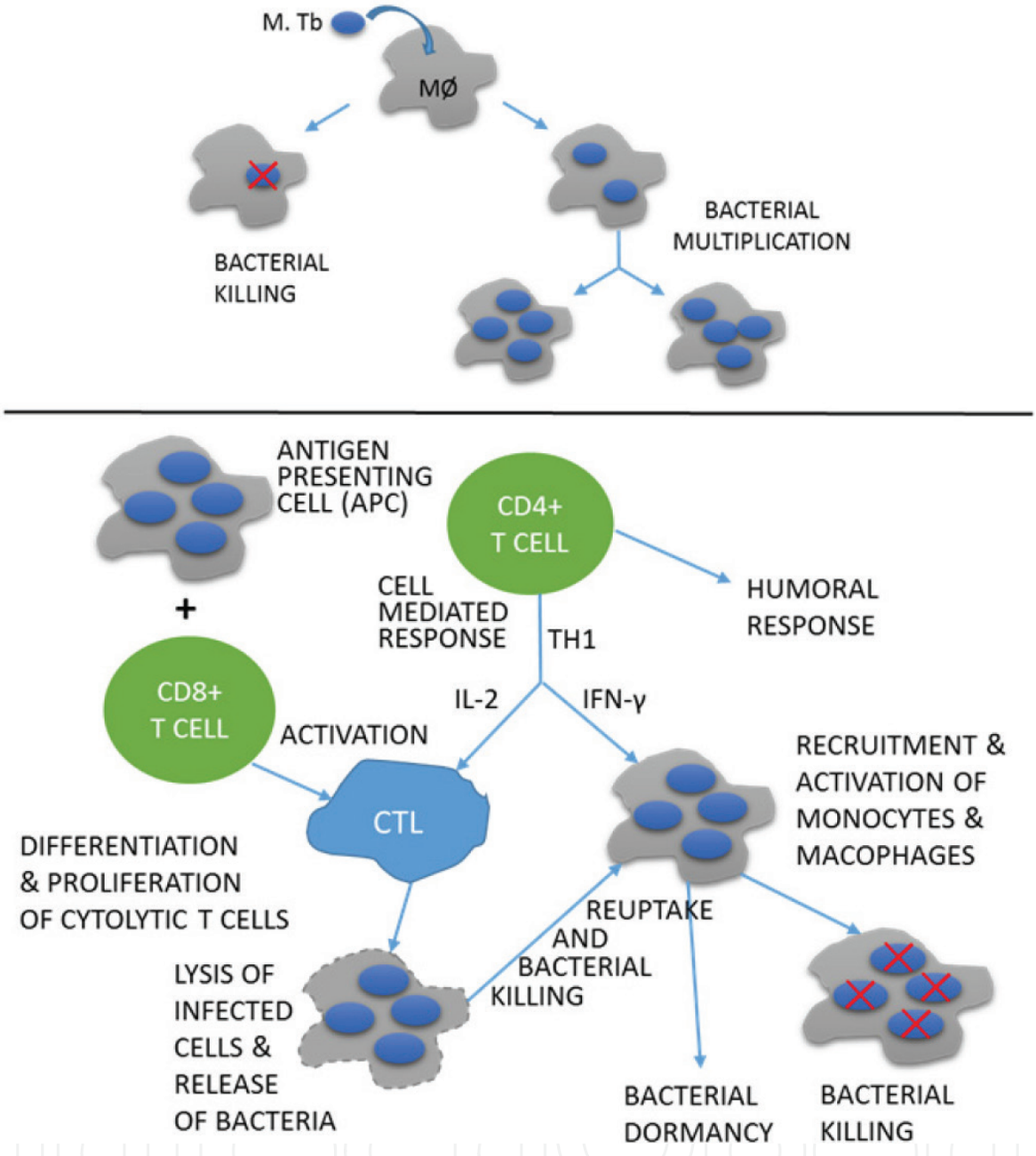

Figure 4. CMI reaction of *M. tuberculosis* [8].

2.3. Immunologic response to *M. tuberculosis*

As intracellular pathogen bacteria, *M. tuberculosis* develops various strategies to be able to survive in the macrophage and form granuloma in the organ of the host. Under the same way, the infected phagocyte cells and the surrounding tissues will respond to the presence of this interfering pathogen. Today, DNA array and proteomic examinations have been used to study the gene expression and the composition of bacterial protein from various strains of *M. tuberculosis* living in different microenvironments. The objective is to study the mechanism of interaction between *M. tuberculosis* and the host.

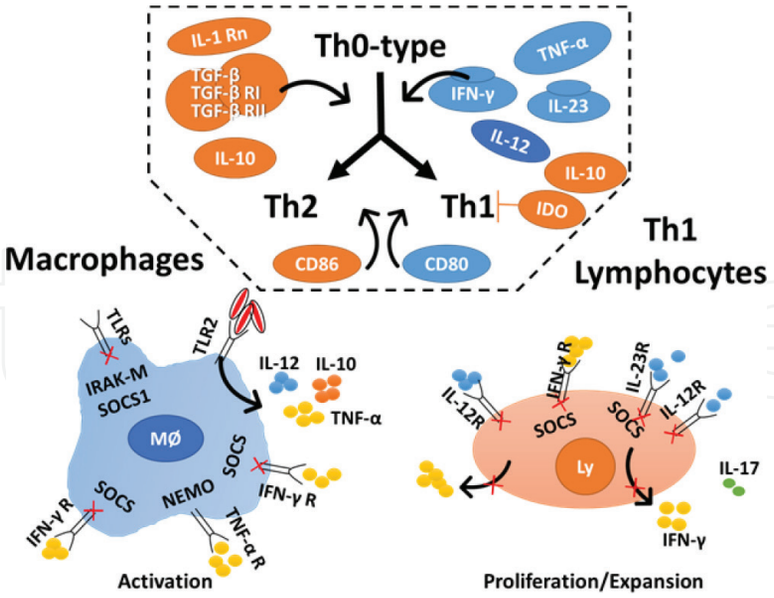

Figure 5. Helper T cells mechanism in *M. tuberculosis* infection [11].

When breathe in droplets containing *M. tuberculosis*, the infectious droplets will be throughout the respiratory tract. The majority of the bacilli in the droplets will be caught in the upper respiratory tract, where the goblet cells secrete mucus. The mucus will catch foreign substrate and the cilia in the cell surface will keep moving the mucus and catch the particles released by the upper respiratory tract. This mechanism provides the body a physical defense system to prevent further infection. The bacteria in the droplets that are managed to go through the mucociliary system and reached the alveolus rapidly will be surrounded and phagocyted by a large number of alveolar macrophages in the alveolus chamber. Macrophage is the next body defense system against *M. tuberculosis* which is able to interfere with the invasion process and prevent infection. After being ingested by the macrophages, *M. tuberculosis* continues to propagate slowly by dividing every 25–32 hours. Regardless, the infection will be controlled or continue to progress, and the initial development of bacterial cells will involve proteolytic enzymes and cytokines produced by the macrophages in order to degrade them. Cytokines produced will attract T lymphocytes and macrophages will then present microbacterial antigen on the surface of T cells. This process of initial defense will continue for 2–12 weeks. *M. tuberculosis* will continue to grow until the number is sufficient to avoid cellular immune response detectable by tuberculin test.

In people with good cellular immune system, the next stage of the body defense mechanism is granuloma formation around the *M. tuberculosis*. The mechanism will generate nodular-type lesion originated from the accumulation of T cells and activated macrophages. The accumulation of the cells creates a microenvironment limiting the replication and the spread of the microbacteria. This environment impaired the macrophages and produce necrosis liquid in the center of the lesion. However, in this condition the *M. tuberculosis* bacilli will still be able to adapt to survive. *M. tuberculosis* can alter the expression of its phenotype, such as the regulation protein to increase the survival. In 2 or 3 weeks, the necrosis

environment will resemble cheese tissue, often called as cheesy necrosis, with characteristics of low oxygen level, low pH, and limited nutrition to limit bacterial growth. Lesion in people with sufficient immune system is usually through fibrosis and calcification that success to control infection that the bacilli will be in the dormant state, and the lesion will then improve. Lesion in the people with insufficient immune system will develop to primary progressive tuberculosis.

3. Laboratory detection to diagnose the infection of *M. tuberculosis*

In this subchapter, the method of laboratory diagnosis of *M. tuberculosis* existence consisting of smear microscopy, culture, genotyping, immunology, and other examination modalities like radiology and histopathology will be discussed.

3.1. Smear microscopy

There are several methods to make accurate diagnosis by using a common smear microscopy: Ziehl Nelseen staining dan auramine staining. Cell morphology observation may be conducted by using a standard light microscope and a fluorescence microscope. Today, light-emitting diodes (LED) microscope has been developed, which is more efficient, consumes low power, and does not require a dark room. However, diagnosis of tuberculosis infection by using smear microscopy has some weaknesses, such as the number of cells per milliliter sample required is quite large (10,000 CFU/mL sample) and it quite often gives negative results, especially in patients with immune system disorder and paucibacillary [12].

3.2. Culture

Culture is a gold standard for tuberculosis diagnosis. This method only needs a relatively small number of germ cells (10–150 CFU/mL sample). Culture method is divided into two, liquid culture and solid culture (egg and agar-based). BACTEC 460TB, MGIT 960, MB/BacT system, MB Redox, and ESP Culture System II are the examples for liquid culture. Lowenstein-Jensen is an egg-based solid medium while Middlebrook 7H10/7H11 is agar-based solid medium. The growth in liquid culture medium is relatively fast compared to the solid culture medium, even though the liquid culture generally cannot be used to determine directly the nontuberculosis species based on the morphology of the colony [13, 14].

3.3. Genotyping

This method is based on the amplification of a specific target gene based on the principles of polymerase chain reaction. Today, it has been applied as a detection method for *M. tuberculosis* directly from the patient's sample, which detects the presence of the suspected bacteria altogether with the resistance to the antituberculosis, rifampicin. This device is called GeneXpert MTB/Rif test and has been endorsed by WHO. Some identification methods of *M. tuberculosis* which are based on the attachment of the target DNA amplification product to the probe of

HAIN Lifescience have also been used, such as Line Probe Assay, GenoType®Mycobacteria Direct assay, and INNO-LiPA MYCOBACTERIA of Innogenetics N.V. Genotyping method has some advantages from the time of examination that is relatively short and accurate. However, this method has some weaknesses such as requiring well-trained operators, presence of inhibitor in the sample, and easily contaminated [15].

3.4. Immunology

3.4.1. Tuberculin skin test (TST)

This test is more common to detect miliary tuberculosis than lung tuberculosis. Tuberculin anergy ranges from 35 to 74% in pediatric and 20–70% in adults. However, positive results of TST do not always indicate active tuberculosis [16].

3.4.2. Interferon-gamma release assay (IGRAs)

This *in vitro* examination is based on the production of gamma interferon by the T cells that may be detected with enzyme-linked immunosorbent assay (ELISA) and enzyme-linked immunospot (ELISPOT). This test will give a good result if applied to pediatric tuberculosis patients, patients that received BCG, and patients with HIV-AIDS. Like TST, IGRAs is also not able to determine the presence of active tuberculosis infection, besides the cost of examination is relatively expensive [17].

Serological examination methods, up until now, are not recommended by WHO to detect tuberculosis infections, both lung tuberculosis and miliary tuberculosis.

3.5. Other diagnostic methods

Some other diagnostic methods have also been used to determine tuberculosis infection, especially in miliary tuberculosis patients, such as ultrasonography, in which method can help detecting ascites, focal hepatic and splenic lesion, intra-abdominal lymphadenopathy, etc. CT and MRI have also been successfully used to detect lesion in the liver, spleen, intestine, and several other inner organs. Whenever possible, tissue biopsy could be done and histopathology examination may then be done by using hematoxylin-eosin staining to see the presence of granuloma and giant cells indicating infection by *M. tuberculosis* [17].

Mycobacterium tuberculosis detection using smear microscopy, genotyping, immunology, and other examination methods cannot confirm the existence of living bacteria in the sample preparation. Culture method is the only one that can assure to find living bacteria in the preparation, but if the culture gives negative result, other examinations do not necessarily give negative results.

4. Bone anatomy and histology

Bone tissue is different from other tissues in the body. Bone is a hard tissue, the main support for the body structure, which is composed of connective tissue and strengthens with continuous calcification process, in which function is controlled by the joint. In this subchapter,

the general anatomy of the bone, bone as organ, and bone as tissue together with the functions will be discussed [18].

4.1. General anatomy of bone

4.1.1. Long bone

Long bone has two parts, diaphysis and epiphysis (**Figure 6**). Diaphysis is the part between proximal and distal ends. The empty part in the diaphysis is called medullar cavity and filled with yellow bone marrow.

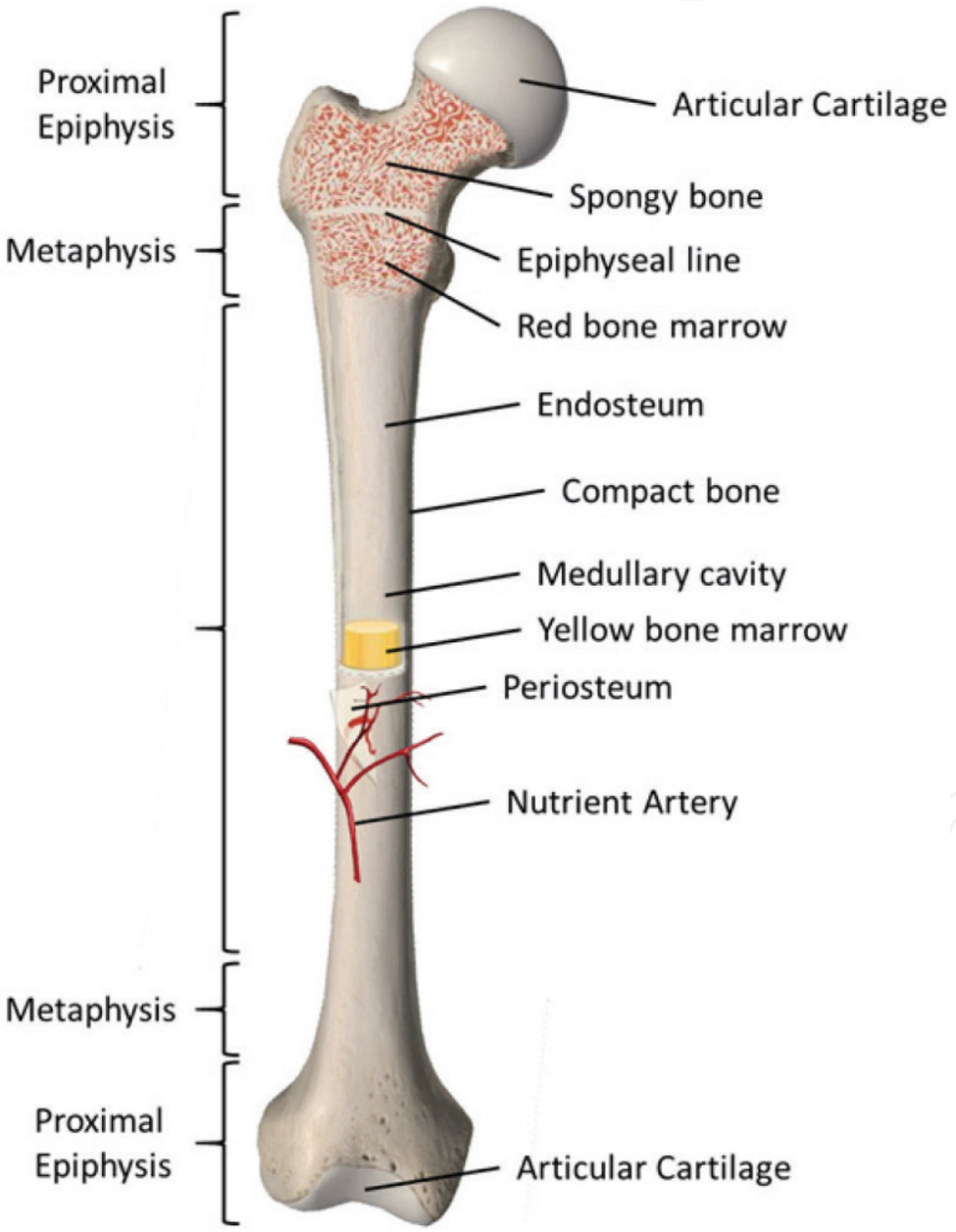

Figure 6. Bone anatomy [19].

The wider part, located near the proximal and distal ends, is called epiphysis, and this part is composed of spongy bone. Red bone marrow filled this spongy bone. Epiphysis and diaphysis meet in metaphysis, a narrow part containing epiphyseal plate (growth plate), which is a hyalin layer (transparent). When the bone stops growing, this cartilage will be replaced with osseous tissue and epiphysis plate will alter to epiphysis line [19].

Medullary cavity has a membrane layer called endosteum, where the bone growth, improvement, and remodeling occur. The outer part of the bone is covered with fibrous membrane called periosteum. Periosteum contains blood vessels, nerves, and lymphatic vessels, providing nutrition to the compact bones. Tendon and ligament also attach to the periosteum covering all bone's outer surface, except the part where epiphysis meet the other bone's end and form a joint. In this part, epiphysis is covered with articular cartilage, a thin layer of cartilage functioned to reduce friction and act as a shock absorber [19].

4.1.2. Cortical bone

Cortical bone is a part of compact bone found below the periosteum and the diaphysis of the long bone, the function of which is to support and protect. Microscopic structure of the cortical bone is called osteon or haversian system. Each osteon consists of concentric ring comprising of calcified matrix called lamellae (or called lamella if single). Down to every osteon is centralis canalis or haversian canal, where blood vessels, nervus, and lymphatic vessels found. These vessels and nervus will be branched in the canal cavity, called Volkmann's canal, and then extends toward the periosteum and endosteum [19].

4.1.3. Spongiosa bone

Like the cortical bone, spongiosa bone (also known as cancellous bone) contains osteocytes inside the lacuna but not arranged in concentric circle (**Figure 7**). Lacuna and osteocytes are arranged in grid-like form called trabeculae (or called trabecula if single). Trabeculae look like a random connection but every trabecula is formed to provide strength to the bone. The spaces in the nets formed by the trabeculae give balance to the compact bone by making the bone total mass lighter that the muscles could move the bone easily. In addition, the cavity

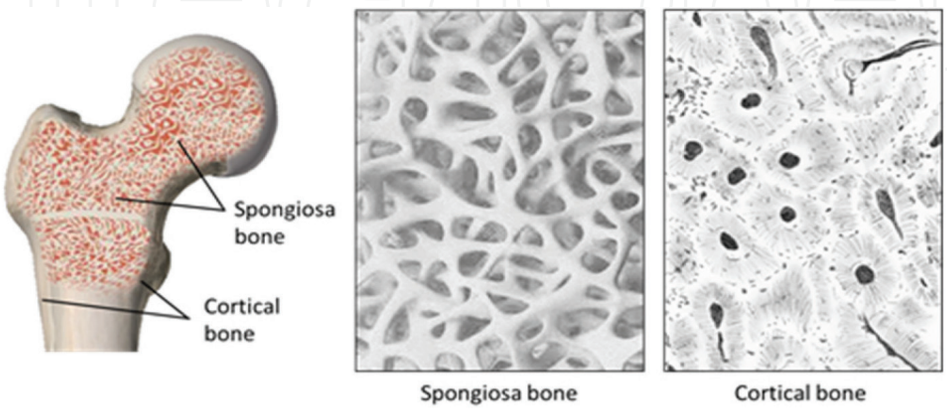

Figure 7. Spongiosa and cortical bone structure [19].

inside the spongiosa bone contains red bone marrow protected in the trabeculae, here the process of hematopoiesis also occur [19].

4.2. Bone as tissue

Bone contains cells that are resided in the collagen matrix that prepare the surface for salt crystals adherence (**Table 2**). These salt crystals are formed when calcium phosphate and calcium carbonate are joined to form hydroxyapatite that will deploy other inorganic salts like magnesium chloride, fluoride, and sulfate toward the collagen fibers. The crystals of hydroxyapatite give strength and rigidity to the bones while the collagen fibers give flexibility. There are four types of cells found in the bone tissue: osteoblast, osteocyte, osteogenic cells, and osteoclast [19, 20].

4.3. Blood circulation in the bone

The circulation system in the bone begins with two entries: from outside to inside (from periosteum to bone medulla) and from inside to outside (from the medulla to periosteum). These systems intersect or meet in the bone cancellous area filled with cavities and in the cortical area filled with haversian canal [21].

The important thing in blood circulation in the bone is that this system, in one part, has already filled with capillary blood vessels, and the other part is flowed by the Arterial system. Generally, capillary blood vessels are the end of the arterial blood vessels, where the cells will be released to the tissue and then charge exchange happens. Oxygen-containing blood will be released to the tissue, and the blood containing CO_2 will enter the vein capillary and so forth, then return to the heart. Other things to consider is that a part of the blood vessel will then end in the medulla wall, which subsequently become the place of exchange of hematopoietic cells where the cells form, develop, and mature. In the bone medulla, regeneration and degeneration occur. Regeneration is a process of bone maturation and degeneration is a process of dead bone destruction [21].

Cell	Function	Location
Osteoblast	Bone formation	Periosteum, endosteum, and at the part of bones growing
Pluripotential stem cell	Differentiated into osteoblast	Inner layer of periosteum and bone marrow
Osteocyte	Maintain the mineral concentration in the matrix	In the matrix
Osteoclast	Bone resorption	Bone surface and in the area that have been wounded

Table 2. Types of bone cells with the function and location.

5. Microenvironment of bone

Microenvironment of the bone is determined by the histologic structure of the bone that it may be divided based on the types of bones, i.e., cancellous bone and cortical bone.

(a) Cancellous bone:

Histologically, cancellous bone has the characteristics to contain many blood vessels and loose intracell chamber to form a hollow structure. This hollow structure enables the blood vessels and red bone marrow to reside there and creating an oxygen-rich environment to increase the metabolism processes and become slightly acidic. This condition is a favorable environment for the growth of *M. tuberculosis.*

(b) Cortical bone:

Histologically, the cortical bone has a more compact structure with more bone cells. With this more bone cells, there will be more bone matrix produced. This liquid bone matrix will attract bone minerals, such as calcium, magnesium, phosphor, etc., to be deposited and make the cells trapped in there. Because of the compact and solid structure of this bone, there are very little blood vessels found creating an oxygen-poor environment, there is only little metabolism and the condition is slightly basic. This becomes a nonfavorable condition for the *M. tuberculosis* growth.

5.1. Living environment

Bone microliving environment is formed by the cells constructing the bone itself and the active cells which periodically interact with the bone cells. Activation of these cells gives impact to the temperature, pH, gas concentration, and liquid concentration, and at the same time trigger calcium, phosphor, and other minerals to be deposited in the bone. This activity of these cells maintain the bone growth, bone strength, endurance against attack, and trigger the bone metabolism process [21–23].

In a condition with low temperature bone macroenvironment, the cells are trying to increase the microtemperature by doing metabolism activities. When the environment is basic, the bone cells will produce CO_2 that will make the microenvironment back to its normal pH. In a condition where the oxygen in the microenvironment of the bone drop as a result of blood vessels obstruction, the bone cells will try to reduce their oxygen consumption in order to be able to maintain the oxygen level. In another condition, like hypoxia, the bone cells will decompose CO_2 to O_2 and CO [23, 24].

In the process of new bone formation, the osteoblast cells produce matrix and release them to the microenvironment that it is needed in a high concentration of calcium and phosphor. This enables the formation of new bone and bone regeneration, like in cases of fracture. For bone strengthening, other inorganic minerals are required to strengthen the bone structure [21, 23, 24].

5.2. Acidity-alkalinity (pH)

Basically, the acidity or alkalinity of the bone microenvironment is determined by comparing the pattern of the acidity-alkalinity of cancellous bone and cortical bone.

There are many blood vessels in the cancellous bone which allow improvement in the metabolism process and oxygen exchange therein resulting in a relatively more acid environment in the cancellous bone than in the cortical bone. This is possible, because logically,

in a condition with a very active metabolism process the exchange in O_2 and CO_2 is very high and so is in other substances, i.e., the products of metabolism that will give acid environment (pH decrease).

There should be a mechanism of the body and the existing system to return the pH to be ideal again when there is a pH decrease. The function to restore and maintain the ideal condition is suspected to be provided by the immune system, such as leucocytes and macrophages.

When compared to the metabolism process in the cancellous bone and in the cortical bone, the metabolism process in the cortical bone is considered as less active. This could be caused by:

(a) The cortical bone environment has a solid character with narrow intercell spaces making the development of the bone cells smaller, which in turn will make the pH in the cortical bone relatively higher or the microenvironment more alkaline.

(b) This situation will be controlled by the immune system that will make the pH of the microenvironment of the cortical bone approximately the same as the cancellous bone.

5.3. Temperature

When the temperature in the microenvironment of the bone is discussed, it means how to create a physiological optimum temperature. Naturally, the condition of normal temperature will be maintained by the body through the thermostat mechanism controlled by the brain.

Temperature is determined from the result of metabolism and chemical mechanism and the interaction among the living cells, for example, between the osteoblast and the immune system, the formation of the calcium, phosphor deposits, etc. In determining the temperature of the microenvironment of the bone, it should differentiate between the temperature outside the cells and the temperature in the intercell spaces. Temperature will be created by the heat rises as a result of chemical reaction, such as:

(a) Biochemical reaction outside the cells producing heat triggered by, for example, H_2O, CO, CO_2, O_2, and other carbon chains.

(b) Reaction inside the cells, both aerobe and anaerobe, producing ATP and releasing heat from the cells.

(c) Metabolism inside the cytoplasm and in the nucleus.

5.4. Gas level in the interstitial chamber

The gases influencing the microenvironment of the bone are oxygen (O_2), carbon dioxide (CO_2), carbon monoxide (CO), nitrogen (N_2), and other gases in small number, where O_2 and CO_2 become the most dominant. The existence of these gases will form particular composition of the interfacial environment of the bone.

In the bone, both gases will exchange in the interstitial chamber, meaning that if O_2 is brought by the red blood cells from the lung and then delivered through the blood vessels to the

tissues, the red blood cells will get into the interstitial chamber and then release the O_2 to the environment. Further, O_2 will be taken up by the bone cells to perform metabolism processes and subsequently, the cells will get energy, produce energy, and metabolite concurrently while releasing CO_2 and O_2 again. In a particular situation, for example, in the condition of poisoning, O_2 will also bring another gas, such as N_2, which physiologically cannot be caught by the red blood cells to be released in the tissues.

5.5. Liquid

Among the bone cells, the osteoblast will produce bone matrix in the form of liquid comprising of protein and mineral salts that will attract calcium, phosphor, and other materials from the environment or metabolite products to be deposited into the matrix.

This deposit will cause solidification that makes the bone structure hard. In the cancellous bone, there are still cavities enabling the interaction among the bone cells (osteoblast, osteocyte, and osteoclast). These interbone cavities are formed when osteocytes trapped by the solidified bone matrix and leaving chambers which still contains liquid that is able to carry nutrition, gas, and important substances like hormones, enzymes, cells, etc., so that the osteocytes are still able to be active [24].

This environment certainly has an ideal concentration, where the composition of the liquid flows in the bone cavities or intermatrix cavities and contribute to the metabolism processes. Based on the above, this microenvironment is largely determined by the protein, blood cells, gas, and mineral transportation.

It is needed to specify the level of protein that could be delivered and form liquid so that it does not disturb the metabolism process, so that the possible ideal concentration of various structures in the bone remains capable of performing activities.

6. Interaction of *M. tuberculosis* and bone microenvironment

The interaction between *M. tuberculosis* and the microenvironment in the bone may be differentiated by the interaction of the bacteria and the nonimmune cells, the interaction of the bacteria and the organic environment, and the interaction of the bacteria and the inorganic environment.

6.1. Interaction of bacteria and nonimmune cells

The interaction between *M. tuberculosis* and nonimmune cells, like the bone cells (osteoblast, osteocyte, and osteoclast), is not mutually destroying or weakening, but this interaction will cause indirect disturbance in the form of metabolism disturbance and bone cell growth disturbance. As an example, the presence of *M. tuberculosis* debris will cause disturbance in the communication in both intercells and cells and the environment in performing metabolism, although it is not yet clear at what level the disturbance occur.

The communication in both interbone cells and the cells and the environment may occur through the following mechanisms:

(a) Direct exchange, the extracellular materials and intracellular materials directly exchange as a result of high difference in the cell wall permeability, for example, in the Na-K pumping.

(b) Indirect exchange, occur through intermediary mechanism that will change the outer and the inner part of the cell wall charges resulting in charge gradient causing the extracellular materials only adhere to the receptor of the outer cell wall.

6.2. Interaction of bacteria and organic environment

The interaction between *M. tuberculosis* and the organic environment is marked with the response of the bacteria to the organic substances in the bone. The organic substances composing the bone are, among others, collagen (bond of protein fibers arranged lengthwise and elastic), polysaccharide protein, and glycosaminoglycan (mucopolysaccharide).

M. tuberculosis will isolate and utilize the proteins from the cell's metabolism products as a medium to grow. The utilization will start with protein denaturation and protein compounds breakdown into simpler compounds, the availability of oxygen supply will create a condition and a new microenvironment that will be used by the bacteria as the media to grow.

6.3. Interaction of bacteria and inorganic environment

Like in the organic environment, the interaction between *M. tuberculosis* and inorganic environment is marked with the response of the bacteria to inorganic substances in the bone.

Inorganic substances making up the bone are, among others, calcium phosphate $Ca_3(PO_4)_2$, i.e., an ionic compound composed of Ca^{2+} ion and PO_4^{2-} ion, and also bicarbonate ion (HCO_3^-). The inorganic substances form a mineral compound called hydroxyapatite ($Ca_{10}(PO_4)_6(OH)_2$) function as a hardening material, provide rigidity, and bone strengthening.

The interaction between *M. tuberculosis* and the bone inorganic environment may be seen as the impact of the structure of the inorganic substance to the bacteria and the way the bacteria utilize the inorganic environment.

Basically, the bone inorganic environment is the respond to the system in the bone. Deposition of calcium and phosphate occur because of the infiltration capacity of the bone cells generating bone matrix. This matrix then attracts calcium and phosphate into the bone structure. The presence of *M. tuberculosis* will affect the condition of the microenvironment by inhibiting the infiltration of calcium and phosphate to the bone and inactivate the bone cells in order to not produce matrix so that the deposition of calcium and phosphate will be disturbed.

Other mechanisms that occur is *M. tuberculosis* colony tries to utilize the inorganic materials in their metabolism by isolating the inorganic matrix into the colony so that brittle bones will generate as a result of calcium and phosphor deposition without matrix.

http://dx.doi.org/10.5772/intechopen.69394

7. Sex hormones' role in *M. tuberculosis* infection

The presence of *M. tuberculosis* in the body happens incidentally. When the bacteria stranded in the droplets enter the respiratory tract and then go into the alveoli and finally spread in the body, *M. tuberculosis* undertake efforts to survive and adapt to the new, continuously changing, environment. This is different with the immune system that always considers the presence of foreign body (including bacteria) as a threat, and macrophage will then come to the location of threat and try to eliminate, isolate, and destroy the foreign body. If the survival of *M. tuberculosis* is not sufficiently high to withstand the immune system attack, an extensive damage in the tissue will occur, and that is the time when the hormonal system will respond by releasing steroid, cortisol, and anabolic hormones aimed to recover the immune system in order that the damage is not getting wider.

Hormonal system is known to be able to strengthen or weaken the immunity system. During the immunity reaction, TNF alpha, interleukin-1, interleukin-6, and interleukin-12 will affect the endocrine system activation through the vascular system. It is also known that hormones can inhibit the proliferation of lymphocytes, cytotoxicity, and strengthen the secretion of IL-2, IL-10, IL-6, and IFN Y.

Sexual hormones have some contribution in the body resistance to tuberculosis infection. At the time of immunity reaction due to *M. tuberculosis* invasion into the body, tissue damage will give feedback to the endocrine gland, this process is known as immuno-endocrine mechanism. This mechanism is a process in which the adaptive and innate immune systems meet the hormone produced by the cytoplasmic endosomes and ended when the cytokines synthesis and T and B cells activities are strengthened. Cytokines, which is an immune response, will mediate and control the process of inflammation, and then influence the endocrine system in order to allow hormonal change [25].

7.1. The role of sexual hormones in tuberculosis infection

7.1.1. Androgen

Androgen has some effects to the cellular and humoral immunities that sometimes this hormone is categorized as an anti-inflammatory hormone, while estrogen will strengthen the humoral immunity and influence the balance of B and T cells. The host's control to *M. tuberculosis* is facilitated through Th1 cells and macrophage cells will be activated.

7.1.2. Testosterone

Testosterone is the main androgen hormone in men with immunosuppressive effect. This hormone disturbs the activation of macrophages and has an important role in lowering the production proinflammatory cytokines, including TNF alpha, and reduce the expression of toll-like monocytes functioning as a pathogen bacteria identifier. Testosterone will reduce the NK cells and induce the production of anti-inflammatory cytokines, such as IL-10, and reduce the production of proinflammatory cytokines, such as TNF, through NF B inhibition.

7.1.3. Esterogen

Esterogen is a proinflammatory hormone stimulating the production of TNF alpha and other proinflammatory cytokines. This hormone also has humoral immunity capability influencing the balance of T and B cells, strengthen the natural killer cells, and prevent immune cells from apoptosis.

7.1.4. Progesterone

Progesterone produced during maternity is suspected to inhibit the development of tuberculosis infection. Progesterone acts as an immunomodulator, which will suppress NK cells and induce IL-4, IL-5, and IL-10, increase the expression of SOCS1, and induce the release of IFN and TNF that will prevent bacteria multiplication.

7.1.5. Estradiol

Estradiol, which is one of maternity hormones, also functions as an immune system activator. Estradiol will strengthen the activity of NK cells through NF B activation; this hormone will induce the production of TNF, IL-1, IL-6, IL-17, and IL-23, and on the other hand, will inhibit the production of IL-4, IL-10, and IL-12.

7.2. The role of endocrine hormones in tuberculosis infection

Immunoendocrine disturbance is related to tuberculosis spread because hormones and cytokines affect the energy release and metabolism. This is especially applied in chronic tuberculosis infection where the pathogen and the immune system are fighting each other for a long time. There are increasing evidences supporting that the stress due to the hormonal alteration could directly stimulate the proinflammatory cytokines production that will then affect the condition related to the disease [26]. Therefore, in imbalance immunoendocrine condition, there will be increase in morbidity and mortality. The role and relation of immunoendocrine in tuberculosis infection can be seen in **Table 3**.

Hormone	Profile and immune response	Hormone concentration during disease	Description
Glucocorticoids	Facilitating Th2 and inducing cytokines production by Th1, IL-12 inducing IFN Y and IL-4	The concentration increases in TB patients when compared to control.	GC has a direct effect to the dendritic cells which increases IL-12 secretion, with less secretion of IL-12 and more secretion of IL-10. The effect of GC in Th2 is reducing the secretion of IL-13,IL-10
DHEA	Reducing the secretion of TGF-B and antagonizes Th2	DHEA concentration decreases by 50% in TB patients	DHEA is permissive to GC inhibition in the cellular immune response, but not in the process of inflammation.

Hormone	Profile and immune response	Hormone concentration during disease	Description
Estrogen	There is shift in Th2 and the secretion of Th1 is reduced, stimulating the synthesis of pro-inflammatory cytokines IL-1, I-6, TNF-a, and inhibit IL-4, IL-10, and IFN-Y	In patients with TB infection, the estrogen level will increase	This hormone will strengthen the humoral immune response and protect the immune cells to apoptosis.
Progesterone	Increasing the progesterone level will inhibit Th1 and produce anti-inflammatory IFNY	In TB patients, the progesterone level will increase	Prevent NFK-B activation & reducing the activity of NK cells
Testosterone	Reducing the expression of IL-4, macrophage, and shift toward Th2 and reducing the secretion of Th1	Testosterone concentration will decrease by 50% in TB patients	Activating the innate immunity. Testosterone will increase the susceptibility to TB infection.
Prolactin	Capable of stimulating the secretion of proinflammatory cytokines	Immune modulator. Increase in prolactin level will result in body weight decrease. It is found increase in prolactin level in TB patient	Stimulating and regulating phagocytosis
Thyroid (T3, T4)	Increasing TNF- α, IL-6, and decreasing TSH	T3, T4 increase in TB patients	Inflammatory cytokines inhibit the thyroid
Growth hormone	IFN- γ inhibit the monocytes phagocytosis	GH level decrease in Patients with TB infection	GH is a human macrophage activator

Table 3. The relation of immunoendocrine in tuberculosis infection and the role in pathogenesis.

8. Soft tissue and hard tissue recovery

M. tuberculosis infection results in hard and soft tissues damage. In this subchapter, the mechanism of the hard and the soft tissues damage in general and the process of recovery of the hard and soft tissues from tuberculosis infection will be discussed.

8.1. Hard tissue (bone)

In the process of infection, there will be struggle of the immune system resulting in tissue damage. In adult patients, osteomyelitis mostly occurs from the direct inoculation and from infection spread from other location. The source of infection may be from direct contamination, iatrogenic contamination during medical procedure, or transmission from contaminated soft tissues. Generally, the development of tuberculosis infection in osteomyelitis is in the form of bacterial invasion, vascular disruption, necrosis, and sequestration [23].

8.1.1. Hard tissue damage

Damage in the bone may be identified through the following:

(a) Bone cells death that will subsequently generate discontinuity or gap among the structure of bones. Immune reaction and *M. tuberculosis* infection will cause obstruction in micro-vascular and resulting in bone cells necrosis.

(b) Bone matrix lysis and denaturation of protein in the bone.

(c) After *M. tuberculosis* is ingested by the macrophage, there will be T cells recruitment. Subsequently, T cells will be activated and produce various cytokines, among others are IL-2 and IFN Y, and then macrophage change into epitheloid cell. The epitheloid cells will combine and form multinucleate giant cells and release lysosomal enzymes resulting in lysis of the surrounding structure of the infection.

(d) Bacterial and tissue debris. The battle between the immune cells and *M. tuberculosis* will generate debris that will be cleaned up by macrophages or join in the caseous necrosis.

(e) Sequester is bone cuts died of vascular disorder.

8.1.2. Hard tissue recovery

There are three items in bone recovery:

(a) The mechanism of the body eliminates the debris through macrophages and immune cells phagocytosis.

(b) The mechanism of sequester and dead tissue decomposition.

(c) The mechanism of debris release through sinus.

At the same time, new bone formation also happens in random order starting from periosteum (peripheral); recovery reaction in the form of hematoma formation also occurs from the middle. Growth factor produced by the stem cells in the periosteum will stimulate the formation of new vascular and nervus that will cover the new bone.

8.2. Soft tissue

Soft tissue is found in almost all over the body. This tissue functions to connect, support, and surround a structure or organ in the body. The types of soft tissue are:

(a) Fat tissue

(b) Muscle tissue

(c) Connective tissue (tendon and ligament)

(d) Synovial tissue

(e) Blood vessel

(f) Lymph tissue

(g) Peripheral nervus

8.2.1. Soft tissue damage

Infection could enter the soft tissue through a torn barrier. When bacteria are in the soft tissue, the macrophage will come and phagocyte the bacteria. Macrophage containing the bacteria will release degradation enzymes and induce the release of cytokines for poly-morphonuclear cells recruitment. Degrading enzymes produced by the macrophages will cause lysis of the cells around the infected soft tissue. Meanwhile, polymorphonuclear cells will trigger further immunity reaction that macroscopically the infected soft tissue will look swelled, suppurated (filled with inflammatory infiltration), and cause pain (due to proinflammatory cytokines release).

8.2.2. Soft tissue recovery

Soft tissue recovery consists of several phases:

(a) Phase of bleeding and inflammatory components recruitment

Inflammatory phase consists of two main phases, early inflammatory and advanced inflammatory phase.

- Early inflammatory phase

In the early inflammatory phase, complement cascade components activation will occur and will be invaded by neutrophil granulocytes (polymorphonuclear) that will fill the wound area in 24–48 hours. The substance in charge of attracting the neutrophils is protein matrix, growth factor, complement, and peptide products from destructed bacteria. Soon the PMN will attach to the endothelium and migrate to the wound area. In the wound area, PMN will phagocytize the bacteria and other foreign substances. Further, PMN also releases enzyme that will lyse and free radicals from oxygen. During this period, the epidermis will increase mitotic activity. In the next 24–48 hours, the epithelial cells in both ends of the wound will migrate and proliferate along the dermis and fill the defect or void components. PMN activity will stop after a few days and the remaining cells will be cleaned up by the macrophages.

- Advanced inflammatory phase

In advanced inflammatory phase, the monocyte cells will fill the wound area. The monocytes will then change into macrophages. The substance attracting the macrophage is the complement, blood coagulation components, immunoglobulin fragments, residual collagen and elastin, and cytokines. Macrophages play many roles in this phase. Besides cleaning the wound area from residual bacteria and tissue, macrophage also secretes growth factor that is functioned to trigger proliferation of extracellular

matrix by the fibroblast, smooth muscle cells, and endothelial cells to stimulate angiogenesis. In this phase, it is already seen the collagen fibers although the fibers have not yet interconnected to each other.

(b) Proliferation phase

This phase occurs after 3 days to 2 weeks. In this phase, fibroblast migration occurs that will produce collagen matrix, hyaluronan, collagen, and proteoglycan. This component is the constituent of extracellular matrix that will support cell growth therein. In this phase, the formation of granulation tissue also occurs. One of the signs of recovery is the formation of granulation tissue. The characteristic of this tissue is pink color, soft, and granulated on the surface. Histologically, this tissue is composed of fibroblast that still continues to proliferate and vascular loop in the collagen matrix that loses. This phase is marked with angiogenesis and new vascular formation (neo-vascularization). In the phase of neo-vascularization, some things will occur: "old" vascular basal membrane degradation to enable the formation of new capillary; endothelial cells migration due to angiogenic cells stimulation; and endothelial cells maturation. The newly formed blood vessels will still swell due to endothelial connection that has not yet been perfect. Granulation tissue can also become a standard to predict wound prognosis. Good tissues will be reddish in color, luminous, hyperemia, and look moist, while the tissue with bad recovery will look soft, brittle, and beefy. A thin layer of epithelium is also formed in this phase and called epiboly. Epithelialization needs humid condition, sufficient nutrition, and free from *Mycobacterium tuberculosis* disturbance.

(c) Remodeling phase

In this phase, collagen synthesis and breakdown happen continuously. Extracellular matrix will undergo remodeling. In this phase, there will also be contraction of wound due to fibroblast and the surrounding extracellular matrix interaction and this process is influenced by cytokines and growth factor such as TGF B, platelet-derived growth factor, and basic fibroblast growth factor. Fibroblast will also produce metalloproteinase that will degrade the collagen. Metalloproteinase depends on zinc to perform its activities.

(d) Wound maturation

This process is the final subphase of the remodeling phase. Fibronectin and hyaluronan will be degraded and the collagen bundle will thicken in line with the increase in tension in the wound. However, this newly formed collagen fibers will not equalize the strength of the previous collagen before the wound.

9. Healing process

If the immune system is not capable of killing the bacteria, the healing process is an additional effort the body does to reduce the *M. tuberculosis* infection. This effort could be done in three mechanisms: bactericide, bacteriostatic, and immunomodulatory by the stem cells.

9.1. Bactericide

Isoniazid and streptomycin have bactericide properties to *M. tuberculosis,* however, the activity of rifampicin is stronger compared to isoniazid and streptomycin both in the lag phase and the log phase [27].

9.1.1. Isoniazid

Isoniazid works by inhibiting the biosynthesis of nicolate (mycolic acid), which is the main component of the cell wall of *M. tuberculosis*. In low concentration, isoniazid will prevent fatty acid chain extension as the first form of mycolic acid. Isoniazid can also remove the acid-resistant property and reduce the number of fat extracted by methanol, by the drug, into the cells.

9.1.2. Rifampicin

Rifampicin is easily absorbed through the gastrointestinal tract. The ester is rapidly hydrolyzed in the bile and catalyzed by esterase in high pH. After 6 hours, all drug preparation will be deacetylated and in the deacetylated form, this drug is still a potent antibiotic. About 6% of the drug excreted through the urine will still be in its initial form, 60% of this drug will be excreted through feces. Rifampicin's half-life is 1.5–5 hours. If consumed with food, the absorption will be inhibited. Drug distribution reach all over the body, even the cerebrospinal liquid. Rifampicin becomes unique for the color make the urine, saliva, tears, and feces red. Rifampicin could be both bacteriostatic and bactericide depending on the concentration. The bactericide activity of rifampicin is obtained through the inhibition of nucleic acid synthesis by inhibiting DNA-dependent RNA polymerase in the subunit B.

9.1.3. Ethambutol

Ethambutol is a bacteriostatic agent that works through the obstruction of cell wall component, i.e., mycolic acid, formation. This drug also inhibits arabinosyl transferase involved in the cell wall biosynthesis. Resistance will easily occur if ethambutol is used alone without combination with other drugs. Ethambutol is well absorbed through the gastrointestinal tract, the bioavailability reach up to 80%, but the penetration to the cerebrospinal liquid is poor. This drug is eliminated through kidney.

9.1.4. Pyrazinamide

Pyrazinamide is an amide derivate from pyrazine-2-carboxylic acid and a nicotinamide analog, and is the third most important antituberculosis drug (OAT) after isoniazid and rifampicin. Pyrazinamide can kill *M. tuberculosis* in the cells in acid environment. The work mechanism is by disturbing the fatty acid synthesis and conversion into pyrazinamidase acid from the tuberculosis bacilli of a semidormant subpopulation in acid environment.

9.2. Bacteriostatic

Bacteriostatic works by preventing and inhibiting bacterial growth, but does not kill them so that bacterial eradication will depend largely on the body's immune system. Isoniazid, rifampicin, ethambutol, and pyrazinamide are first-line anti-tuberculosis drugs having bacteriostatic activity that is almost the same with *M. tuberculosis*, except that the bacteriostatic activity of isoniazid depends on the phase of growth. In the bacteriostatic condition, the host's self-defense mechanism, such as phagocytosis, and antibody production usually will impair the bacteria; in other words, the inhibition of bacterial growth is conducted by utilizing the immune system of the body [28].

9.3. Immunomodulatory by stem cells

Immunomodulatory properties of stem cells are reported to be in the T cells proliferation using a kind of stimuli, including mitogen, CD3/CD28, dan alloantigen. The relation of mesenchymal stem cells and proliferation inhibition of T cells is already known, among others, by reducing the expression of activator marker like CD25, CD38, CD69 in PHA lymphocyte, suppressing the proliferation of CD4 and CD8 [29, 30].

Immunomodulation capability of stem cells seems to rise before the secretion of IL-2 because the antiproliferation effect in mitogen induced by periphery lymphocytes may be repeated by adding IL-2. Further study showed that the mesenchymal stem cells supernatant does not have any role in inhibiting SPM proliferation, but in an *in vitro* experiment by using semipermeable membrane (in order that SPM and leucocytes separated) it is proven that there are soluble factors that can penetrate the membrane and have role in the proliferation suppression. Among the soluble factors produced by the mesenchymal stem cells are prostaglandin E2, IL-10, and hepatic growth factor. The factors proven could suppress the antigen response mediated by T cells. It is also proven that the induction of indolamine 2,3-dioxygenase by the mesenchymal stem cells will stimulate IFN Y. Therefore, the inhibition of mesenchymal stem cells to the proliferation of T cells could be due to tryptophan depletion [29].

The mesenchymal stem cells also have a role in molecular bond programming cell death (PD-1) and the ligand PD-L1 dan PD-L2 that resulted in the inhibition of T cells proliferation through direct contact between mesenchymal stem cells and target cells. The mesenchymal stem cells also increase CD4 and CD25 in cells and proved to have inhibition effect to proliferation and secretion of B cells IgG. When mesenchymal stem cells that are isolated from bone marrow and B cells extracted from periphery blood are cultured together, the result is inhibition of B cells proliferation and immunoglobulin formation due to soluble factors.

Mesenchymal stem cells also interact with dendritic cells by inhibiting the proliferation of monocytes into dendritic cells by also inhibiting the maturation of dendritic cells. Immature dendritic cells will alter the energy of T cells. Mesenchymal stem cells also proved to alter the cytokines secretion of the dendritic cells, such as IL-10, and reduce the regulation of inflammatory cytokines, such as IFN Y and IL-12 dan TNF alpha.

10. Conclusion: key results

The journey of *M. tuberculosis* to the microenvironment of the bone occurs through various environments, which tests the survival of *M. tuberculosis* itself. Generally, microenvironment may be classified as living environment, organic environment, and inorganic environment. *M. tuberculosis* has an extraordinary capability to survive, in responding to the environment threatening its life, by controlling the surrounding environment and adapting to the environment by transforming itself into dormant state or by inactivating all metabolisms.

Acknowledgements

The images and figures in the book chapter were made by the authors themselves.

Author details

Rahyussalim Ahmad Jabir[1,2]*, Andriansjah Rukmana[2,3], Ifran Saleh[1,2] and Tri Kurniawati[4]

*Address all correspondence to: rahyussalim71@ui.ac.id

1 Department of Orthopedic and Traumatology, Faculty of Medicine, Universitas Indonesia, Jawa Barat, Indonesia

2 Dr. Cipto Mangunkusumo General Hospital, Jakarta, Indonesia

3 Department of Clinical Microbiology, Faculty of Medicine, Universitas Indonesia, Jawa Barat, Indonesia

4 Stem Cells Medical Technology Integrated Service Unit, Dr. Cipto Mangunkusumo General Hospital-Stem Cells and Tissue Engineering Cluster, IMERI Faculty of Medicine, Universitas Indonesia, Jakarta, Indonesia

References

[1] Issar S. *Mycobacterium tuberculosis* pathogenesis and molecular determinants of virulence. Clinical Microbiology Reviews, American Society for Microbiology. 2003;**16**(3):463-496

[2] Schlossberg D. Tuberculosis and Non tuberculous Mycobacterial Infections. 5th ed. HillASM Washington DC; 2006

[3] Daniele S, Francesco P, Bruno V, Giuseppe T, Francesco B. The role of bone microenvironment, vitamin D and calcium. Prevention of Bone Metastases. (Eds) M.Gnant 2012;**9**:233

[4] Molecular Expressions Cell Biology and Microscopy Structure and Function of Cells and Viruses. Available from: https://micro.magnet.fsu.edu/cells/bacteriacell.html

[5] Karakousis P, Bishai W, Dorman S. *Mycobacterium tuberculosis* cell envelope lipids and host immune response. Cellular Microbiology. 2004;**6**(2):105-116

[6] Timothy B, Vladyslav N, Preya V, Francis D. Associations between *Mycobacterium tuberculosis* strain and phenotypes. Emerging Infectious Disease. 2010;**16**(2):272-280

[7] Joseph K, Remold HG, Hardy K. Virulent *Mycobacterium tuberculosis* strains evade apoptosis of infected alveolar macrophages. Journal of Immunology. 2000;**164**:2016-2020

[8] Ernst RK, Guina T, Miuer SI. How intracellular bacteria survive: Surface modifications that promote resistance to host innate immune responses. The Journal of Infectious Diseases. 1999;**79**(Suppl 2):S326-S330

[9] Marina AF, Laura LK, Andrea G, Julis S, et al. Virulence factors of the *Mycobacterium tuberculosis* complex. Virulence. 2013;**4**(1):3-66

[10] Dietrich J, Doherty M. Interaction of *Mycobacterium tuberculosis* with the host: Consequences for vaccine development. Journal Compilation APMIS. 2009;**117**:440-457

[11] Barnes P, Wizel B. Type 1 Cytokines and the pathogenesis of tuberculosis. American Journal of Respiratory and Critical Care Medicine. 2000;**161**:1773-1774

[12] Senol G. Laboratory diagnosis of tuberculosis – latest diagnostic tools. Tuberculosis - Current Issues in Diagnosis and Management. Intech, 2013

[13] Francesca B, Roberta F. Reliability of the MB/BacT system for testing susceptibility of *Mycobacterium tuberculosis* complex isolates to antituberculous drugs. Journal of Clinical Microbiology. 2000;**38**(2):872-873

[14] Carroll P, Schreuder LJ, Muwanguzi-Karugaba J, Wiles S, Robertson BD, Ripoll J, et al. Sensitive detection of gene expression in mycobacteria under replicating and non-replicating conditions using optimized far-red reporters. PLoS ONE. 2010;**5**:e9823. DOI: 10.1371/journal.pone.0009823

[15] Neonakis IK, Gitti Z, Baritaki S, Petinaki E, Baritaki M, Spandidos DA. Evaluation of GenoType mycobacteria direct assay in comparison with Gen-Probe *mycobacterium tuberculosis* amplified direct test and GenoType MTBDRplus for direct detection of *Mycobacterium tuberculosis* complex in clinical samples. Journal of Clinical Microbiology. 2009;**47**(8):2601-2603

[16] Rose DN, Schechter CB, Adler JJ. Interpretation of the tuberculin skin test. Journal of General Internal Medicine. 1995;**10**(11):635-642

[17] Mori T. Usefulness of interferon-gamma release assays for diagnosing TB infection and problems with these assays. Journal of Infection and Chemotherapy. 2009;**15**:143-145

[18] Sharma SK, Mohan A, Sharma A, Mitra DK. Miliary tuberculosis: New insights into an old disease. The Lancet Infectious Diseases. 2005;**5**:415-430

[19] Iwaniec UT, Wronski TJ, Turner RT. Histological Analysis of bone. Methods in Molecular Biology. 2008;**447**:325-341. DOI: 10.1007/978-1-59745-242-7_21

[20] Timothy RA. Acid-base regulation of bone metabolism. International Congress Series Elsevier. 2007;**1297**:255-267

[21] Ryan ET, Matthew JS. Skeletal blood flow in bone repair and maintenance. Bone Research. 2013;**1**(4):311-322

[22] Rahyussalim AJ, Tri K, Ismail, Errol U. H, Nuryati C. N, Andriansjah R. New bone formation in tuberculous infected vertebral body defect after bone marrow stromal cells administration in rabbit model. Asian Spine Journal. 2016;**10**(1)

[23] Nair SP, Meghji S, Wilson M, Reddi K, White P, Henderson B. Bacterially induced bone destruction: Mechanism and misconception. Infection and Immunity. 1996;**64**(7): 2371-2380

[24] Debra LP, Ali K, Nancy AB. Growth of *Mycobacterium tuberculosis* in a defined medium is very restricted by acid pH and Mg^{2+} levels. Infection and Immunity. 2000;**68**(8):4518-4522

[25] Garcia-Gomez E, Gonzalez-Pedrajo B, Camacho-Arroyo I. Role of sex steroid hormones in bacterial-host interactions. BioMed Research International. 2013;**2013**:1-11

[26] Jyothi PM, Rajashekar M, Sumanlatha G. Role of Immuno-Endocrine interactions in tuberculosis. Donnish Journal of Infectious Diseases and Immunity. 2015;**1**(1)

[27] Yew WW, Leung CC. Antituberculosis drugs and hepatotoxicity. Respirology. 2006; **11**(6):699-707

[28] Chelluri L, Chelluri PE, Vennila P, Gokhale A, Adavi V. Preliminary report on immunomodulation of mesenchymal stem cells in M.tb infection. The Internet Journal of Infectious Diseases. 2009;**8**(1):1-4

[29] Rahyussalim AJ, Tri K, Andriansjah R. *Mycobacterium Tuberculosis* contaminant risk on bone marrow aspiration material from iliac bone patients with active tuberculosis spondylitis. BioMed Research International. 2016;**2016**. Article ID 3852940, http://dx.doi.org/10.1155/2016/3852940

[30] Rahyussalim AJ., Andriansjah R, Kusnadi Y, Ismail HD, Lubis AM, Kurniawati T, Merlina M. Effect of *Staphylococcus aureus* and *Staphylococcus epidermidis* debris on bone marrow stromal cells growth. Acta Medica Indonesiana. 2012;**44**(4):304-911

IntechOpen