THE DETERMINATION OF CHIMERIC SECRETORY IGA: TB EPITOPES IN GOAT MILK

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by

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TABLE OF CONTENT

LIST OF TABLES

LIST OF FIGURES

LIST OF ABBREVIATIONS

APCs	Antigen presenting cells
Acr	Latency-associated antigen
BCG	Baccilus Calmette-Guerin
C	Celsius
dIgA	Dimeric immunoglobulin A
EPTB	Extra-pulmonary Tuberculosis
Fab	Antigen binding fragment
Fc	Crystallize fragment
g	Gram
IBM	International Business Machines
IFN	Interferon
IgA	Immunoglobulin A
IgM	Immunoglobulin M
IL	Interleukin
ImageJ	Java image processing program
kDa	kiloDalton
mAgm	Multiepitope seq-85-murine IgA
MDR	Multi drug resistance
mg	Miligram
ml	Mililitre
mM	Milimolar
Mtb	*Mycobacterium tuberculosis*
PDVF	Polyvinylidene fluoride

pIgA	Polymeric immunoglobulin A
pIgR	Polymeric immunoglobulin receptor
PRR	Pattern recognition receptor
PTB	Pulmonary Tuberculosis
Rpf	Resuscitation promoting factor
rpm	Revolutions per minute
SC	Secretory component
SDS-PAGE	Sodium dodecyl sulfate polyacrylamide gel electrophoresis
S.E.M	Standard error of the mean
sIgA	Secretory immunoglobulin A
SPSS	Statistical package for the social sciences
TB	Tuberculosis
TGF	Transforming growth factor
TNF	Tumor necrosis factor
USM	Universiti Sains Malaysia
µl	Microlitre
V	Volt
WHO	World Health Organisation

PENENTUAN IGA REMBESAN CHIMERIK: EPITOP TB DALAM SUSU KAMBING

ABSTRAK

Tuberkulosis (TB) pada dasarnya mempunyai dua jenis: jangkitan aktif dan terpendam. Vaksinasi semasa terhadap TB, iaitu Bacillus Calmette-Guerin (BCG), tidak dapat memberikan perlindungan dengan keberkesanan yang tinggi terhadap TB aktif sambil menunjukkan tiada perlindungan terhadap jangkitan terpendam. Pemberian vaksin BCG adalah melalui sistemik. Oleh itu, penciptaan vaksin tuberkulosis mukosa dengan tumpuan kepada juzuk protein telah menjadi bidang minat penyelidikan. Pembangunan vaksin mukosa adalah sukar kerana sifat sistem mukosa. Dalam kajian sebelumnya, kumpulan kami membangunkan vaksin mukosa dengan menggabungkan tiga epitop TB yang berbeza, iaitu Ag85b, protein yang berkaitan dengan latensi (Acr), dan faktor penggalak resusitasi (Rpf) dari *Mycobacterium tuberculosis* (Mtb), yang dikaitkan dengan sekretori immunoglobulin A (sIgA). Binaan ini telah diklon ke dalam genom vektor virus yang berkaitan dengan Adeno sebelum ditransduksikan ke dalam kelenjar susu kambing hamil. Acr dan Rpf adalah komponen penting yang terlibat dalam pengaktifan semula Mtb yang tidak aktif. Bersama-sama dengan Ag85b, mereka telah menjadi sasaran penting untuk pembangunan vaksin mukosa TB baharu dengan matlamat untuk mencegah pengaktifan semula basilli tuberkel tidak aktif yang dikaitkan dengan tuberkulosis terpendam. Dalam kajian ini, ekspresi epitop immunodominan dalam susu kambing dinilai menggunakan tompok barat dengan antibodi Rb pAb ke *Mycobacterium tuberkulosis* Ag85b. Hasilnya menunjukkan bahawa band pada kira-kira 75 kDa dikesan, menunjukkan bahawa epitop berjaya dibuktikan dalam susu. Ekspresi

protein chimerik dalam kepekatan susu kambing yang berbeza adalah nyata pada $p < 0.05$.

THE DETERMINATION OF CHIMERIC SECRETORY IGA: TB EPITOPES IN GOAT MILK

ABSTRACT

Tuberculosis (TB) basically has two types: active and latent infections. The current vaccination against TB, which is Bacillus Calmette-Guerin (BCG), is unable to provide protection with high efficacy against active TB while showing no protection against latent infection. The administration of BCG vaccination is systemic. Thus, the creation of mucosal tuberculosis vaccines with a focus on the protein constituent has become an area of research interest. The development of mucosal vaccines is difficult due to the nature of the mucosal system. In a previous study, our group developed the mucosal vaccine by combining the three different epitopes of TB, namely Ag85b, latency-associated protein (Acr), and resuscitation-promoting factor (Rpf) from *Mycobacterium tuberculosis (*Mtb), linked with secretory immunoglobulin A (sIgA). The construct has been cloned into the genome of the adeno-associated viral vector before being transduced into the mammary gland of the pregnant goat. Acr and Rpf are important components that are involved in the reactivation of dormant Mtb. Together with Ag85b, they have become important targets for the development of a new TB mucosal vaccine with the goal of preventing the reactivation of dormant tubercle bacilli associated with latent tuberculosis. In this study, the expression of the immunodominant epitope in the milk of the goat was evaluated using western blot with Rb pAb to *Mycobacterium tuberculosis* Ag85b. The result shows that a band at approximately 75 kDa was detected, indicating that the epitopes were successfully expressed in milk. The expression of chimeric proteins in different concentrations of goat milk is significant at $p < 0.05$.

CHAPTER 1

INTRODUCTION

1.1 Background study

TB caused by Mtb, is one of the leading causes of death in the world. When a person with an infectious disease coughs, sneezes, or spills, the infectious agent enters the air and has the potential to spread to other people. The one piece of positive information regarding this pathogen is that it may be prevented and treated with medication as well as a vaccination. Unfortunately, the prevalence of this disease is rather high. In areas with lower incomes, the prevalence of TB is disproportionately higher than in wealthier areas. The World Health Organisation believes that between 5% and 10% of people who are infected with Mtb will develop the symptoms of tuberculosis disease and will eventually pass away because of the illness (WHO, 2023). Carriers of the disease who do not exhibit symptoms are unable to pass it on to others because they are immune to its effects.

In addition, the disease can frequently be treated with antibiotics. If treatment is not provided, there is a chance that this sickness will result in death. According to Coppola and Ottenhoff (2018), more than 10 million people are given a diagnosis of active tuberculosis each year, and nearly two million people pass away because of the disease. People who are infected with tuberculosis have a chance of developing either the clinical state known as latent tuberculosis infection, which is not infectious, or the clinical condition known as symptomatic active tuberculosis, which

is infectious. There are several symptoms of tuberculosis, some of which include persistent fatigue, loss of appetite, weight loss, and nighttime sweats.

Vaccine is essentially a substance that increases a person's resistance to a particular disease. Vaccinations are becoming more prevalent. In contrast to the traditional antibacterial treatments used to treat tuberculosis, which must be taken for an extended period and rigorously adhered to by the patient, this treatment does not require the patient to adhere to any restrictions. Vaccines today carry a lower risk of side effects than they did in the past, and widespread vaccination campaigns can effectively prevent the spread of infectious diseases.

As a result, a new class of anti-TB immunisations is produced using killed bacilli or subcellular derivatives (Barclay *et al.*, 1967). However, these immunisations often cause a non-specific inflammatory response rather than long-term protection (Orme, 1988). Although secretory vaccines have been found to be highly protective in animal experiments, it is possible that the complete protein repertoire will lead to antigen presentation competition, resulting in a subpar immune response. Not only is the recipe difficult to duplicate, but it may also contain harmful ingredients. Therefore, researchers have been putting in long hours to produce vaccines that are both extremely effective and safe for use on a global scale. The WHO released its annual tuberculosis report in August 2019, claiming that four new anti-TB vaccines are currently being investigated in clinical trials around the world (WHO, 2019). These include viral carriers, proteins or adjuvants, mycobacteria entire cells or extracts, and mycobacteria-based live vaccines. Until now, only the protein subunit vaccine has shown significant promise in overcoming the most serious issues with traditional methods, such as the risks of using live or attenuated microorganisms with

potentially toxic viral vectors and the possibility of bacteria reproducing or becoming dangerous again (Duong *et al.*, 2023).

Gavi (2020) claimed that the protein subunit vaccine has a few benefits over other types of vaccines mentioned above, such as the fact that it is a well-tested technology. This is because there has been a lot of research in vaccines for a long time, making it easy to develop and check the quality of the protein subunit vaccines. Aside from that, it is thought to be safe for people with weak immune systems because it is not likely to cause the disease they are trying to prevent (Gavi, 2020). Other than that, this protein subunit vaccine does not contain any live pathogens. Instead, it is made up of purified protein or protein fragments, which makes it less likely to break down or lose its effectiveness while being stored and distributed (Gavi, 2020). So, it can be said that the development of protein subunit vaccines is essential because it provides a safe and effective way to protect against TB worldwide.

Thus, our novel protein subunit mucosal vaccine is currently being developed by taking advantage of the abundance of secretory IgA within the mucosal system. The TB epitopes from Mtb, such as Acr, resuscitation promoting factor Rpf, and Ag85b, linked with the sIgA, have the potential for protection against both active and latent TB, which has become the goal for this novel vaccine to be developed with high efficacy as compared to current vaccination with BCG against tuberculosis.

1.2 Problem statement

A significant number of pathogens can flourish on the mucosal surface. Once the pathogens can infiltrate the circulatory system, there is a high chance that they will spread to other parts of the body. But most infections, like Mtb, which causes tuberculosis disease all over the world, show up on the mucosal surfaces, where they can do a lot of damage when breathed in. Besides, the current BCG vaccine for tuberculosis provides inadequate protection at the mucosal surfaces, which are the main site of entry for the infection. Instead, it focuses on systemic levels. This vaccine is also not able to protect against latent TB infections, whereas these infections will become active once the immune system has weakened. Thus, this kind of protection is not enough with the main aim of reducing the mortality rates involving tuberculosis disease, especially in low-income countries where there are limited sources for treatment.

There are actually a few compelling pieces of evidence that support the idea that the mucosal immune system can protect against both naturally occurring diseases and vaccinations given through the mucosal route. The conjunctiva, mammary glands, upper airways, middle ear cavity, and epithelium surfaces of the respiratory and intestinal tracts contain lymphoid follicles. Other than that, organised lymphoid follicles can also be found in the genital canal. There is a little evidence suggesting mucosal vaccination can increase significant sIgA, serum antibodies, and cellular immunological responses (Kaul and Ogra, 1998). The examples include mucosal administrations of live attenuated polio vaccine (OPV), rubella virus vaccine known as RA 27/3, adenoviruses, influenza A virus, rotavirus, cholera, and salmonella (Kaul and Ogra, 1998). When a person is vaccinated through the mucosal route, the body

will release a variety of integrins and cell adhesion molecules that are necessary for immune responses. The ability to mount a mucosal immune response protects against not just new illnesses but also recurrent infections. Therefore, it is desirable to develop a vaccine that can also induce pathogen-specific antibodies or cellular immunity directly on the mucosal sites. Not to mention the abundance of secretory IgA within the mucosal system, which is one of the targets for the novel mucosal vaccine to be developed.

In addition to this, the current tuberculosis vaccination involves getting an injection, which some individuals may find to be an unpleasant experience. It has been suggested that innovative mucosal vaccinations be produced to alleviate the pain that is often associated with getting injections. Research on the immunological environment of the skin found that the human skin contains many antigen-presenting cells (APC), which offer a promising channel for vaccination and lead to powerful immune responses only when using modest vaccine doses (Hernandez-Franco *et al.*, 2021). It has been the focus of a significant amount of research and development effort to simplify the process of injecting vaccines under the skin by developing tools that are efficient, risk-free, and simple to operate to stimulate the production of the most robust immune responses possible (Beaujean *et al*., 2023). To tackle the high death rate associated with tuberculosis, especially in nations with a high burden of the illness, intradermal administration of tuberculosis vaccinations has seen widespread use.

Historically, Benjamin A. Rubin recommended using a bifurcated needle to deliver the immunisation, which ultimately resulted in the elimination of smallpox virus infections (Artenstein, 2014). This is the very first event of its type, a vaccination

drive. This method of diagnosing tuberculosis via intradermal injection has been superseded by the Mantoux method, which was established by Charles Mantoux. The Mantoux method is currently considered to be the gold standard. Vaccinations against rabies and BCG are being carried out with its assistance at the present time (Bricks, 2004). After the skin has been stretched, the Mantoux procedure involves inserting hypodermic needles of either a 26 or 27 gauge into it at an angle of between 15 and 20 degrees with the bevel facing up. On the other hand, the various limitations and negative consequences associated with this intradermal injection make it impractical to use.

Eventually, the current parenteral BCG vaccination for tuberculosis will entail the injection of the vaccine into a person, which is more expensive than mucosal immunisation for a variety of reasons. One reason for this is that vaccine administration is difficult and necessitates the use of special equipment such as syringes, needles, and vials. They also must be sterilised, adding to the cost of this parenteral vaccination. Even minor temperature variations during storage, transportation, and distribution can impair the effectiveness of this parenteral tuberculosis vaccine. To lessen the chance of complications after immunisation, the injection may need to be administered by well-trained staff such as a doctor, nurse, or medical assistant. It is critical to have appropriately trained staff on hand to reduce complications and waste (Al Jarad *et al.,* 1999). People may also need to visit the hospital or clinic repeatedly as they may require more than one dose or booster to complete the immunisation. The mucosal vaccination, on the other hand, can be administered through non-invasive methods such as nasal sprays, oral drops, or inhalation, which saves money and simplifies the administration process. This then reduces the demand for medical personnel and the associated expenditures. Thus, it

is best to stick with the goal of mucosal vaccination development to eliminate these unnecessary issues.

It is worth mentioning that the weakness in developing the mucosal vaccination against TB is hampered mainly due to the lack of methods that can ensure the stability of the formulations within the harsh environment of the digestive tract and mucosa. Thus, the development of this mucosal vaccination against TB takes advantage of the immune physiology of secretory IgA that is abundantly present within the mucosal system.

In conclusion, the development of a mucosal vaccination against tuberculosis has the potential to improve protection on both the mucosal and systemic levels. This can be accomplished through the generation of pathogen-specific antibodies or cellular immunity directly at the mucosal sites. In addition, mucosal vaccination can eliminate the difficulties of pain associated with injections as well as discomfort experienced by the recipient, making it easier to use than traditional immunisation methods. Finally, mucosal vaccination has the potential to circumvent the expensive costs that are associated with the conventional parenteral vaccination method. As a result, it is more financially prudent for the researchers to work on developing this method.

1.3 Significant of study

The previous study involved the cloning of protein subunits consisting of Ag85b, Acr, and Rpf from Mtb, along with sIgA, into the genome of the adeno-associated viral vector before being expressed in the HEK cell line. The production of recombinant Adeno associated viral vector (AAV) requires three phases, including

AAV acquisition, AAV harvesting, and AAV quantification, before the AAV vector is transduced into the mammary gland of the goat serving as the bioreactor. After a few days of co-transduction, additional research will consist of detecting the expression of chimeric proteins in goat milk. Thus, it is important to ensure the quality of the milk produced by the mammary gland of the goat to express the chimeric IgA: TB epitopes as planned by the previous study by using the freeze-dried milk provided as well as its purified protein.

Since parenteral vaccination can be difficult and requires specially trained people, it may be worthwhile to investigate alternate delivery routes that introduce vaccine directly into the mucosal surface. The use of the goat mammary gland as a bioreactor lends unique importance to this study in the quest to create innovative mucosal vaccinations. The primary contribution of this research is a better appreciation of the value of the mucosal route in vaccination for boosting local and systemic immune responses while taking advantage of secretory IgA within the mucosal immune system. This type of vaccination can elicit both types of immune responses since it kicks off with a local immunological response. The significance of this study within society is that it can improve the understanding of both immune responses with the aim of reducing the mortality rates related to tuberculosis disease. The mucosal surface is often the first place where tuberculosis symptoms appear. Knowing the importance of a vaccination that offers main site protection is so essential.

Most infections, like Mtb, enter the body through the mucosal surfaces of the respiratory tract, digestive tract, and genitourinary tract. By introducing the vaccine within this mucosal surface, it is possible to induce a significant local immune response that includes sIgA antibodies, mucins, defensins, and other antimicrobial

components. With the advantages of the abundance of sIgA within the mucosal system, it has become the target of this study to develop a novel mucosal vaccine. Besides inducing a local immune response, it can also stimulate the systemic immune response when activated immune cells from mucosal locations travel to distant lymphoid organs like lymph nodes and transmit antigens to other immune cells. This triggers an immunological reaction throughout the body, including the development of antibodies in the circulation and the activation of T-cell responses. It follows that the results of this study can help us better appreciate how protection offered at the primary site of manifestation can be even more effective thanks to mucosal immune responses.

In addition, this research is economically valuable since it contributes to the development of innovative mucosal vaccines, which are cheaper than parenteral immunisation. However, the goat's mammary gland is used as the bioreactor in this study, which eliminates the requirement for costly complex formulations or adjuvants. An alternative to injecting vaccines into a human body could be provided by this type of immunisation. Mucosal vaccination, in contrast to injection, does not necessitate the use of needles, syringes, or any other special equipment, as well as the expertise of medical professionals. As tuberculosis is so prevalent in low-income nations, this has the potential to cut down on patient mortality. Thus, this mucosal vaccination can be seen as more cost-effective than the standard parenteral immunisation for tuberculosis.

Finally, this research is important because it could improve health and productivity and lower tuberculosis death rates, particularly in high-burden nations. As was previously indicated, current parenteral immunisations are expensive to produce,

store, and distribute, which contributes to high tuberculosis death rates in low-income countries. As a result, their death rates may continue to be significantly higher than those in high-income nations. Thus, these countries may benefit from the development of a novel mucosal vaccination by using the mammary gland of the goat as the bioreactor, which could eventually lead to the eradication of the illness in people. This novel vaccine which includes the Acr and Rpf can provide the protection on both latent and active tuberculosis worldwide.

In conclusion, with the goal of reducing and, ideally, eliminating mortality rates associated with tuberculosis worldwide, this study has important societal, economic, and national implications. It is cheaper and easier to scale up while educating people on the significance of both local and systemic immune responses in clearing the body of infections. Finally, it has the potential to lower mortality rates in low-burden countries where tuberculosis infection is nevertheless widespread, especially for the latent infection of tuberculosis.

1.4 Objectives of study

The objectives of the study are:

1. To determine the expression of chimeric secretory IgA: TB epitopes in milk of pregnant goat by Western Blot.
2. To compare the band intensity representing the chimeric secretory IgA: TB epitopes through statistical analysis.

1.5 Flowchart of the study

This study consists of a few stages, including the sample preparation for the milk of the pregnant goat. The samples were prepared by mixing the freeze-dried milk powder with distilled water. Figure 1.1 shows the summary of the methods.

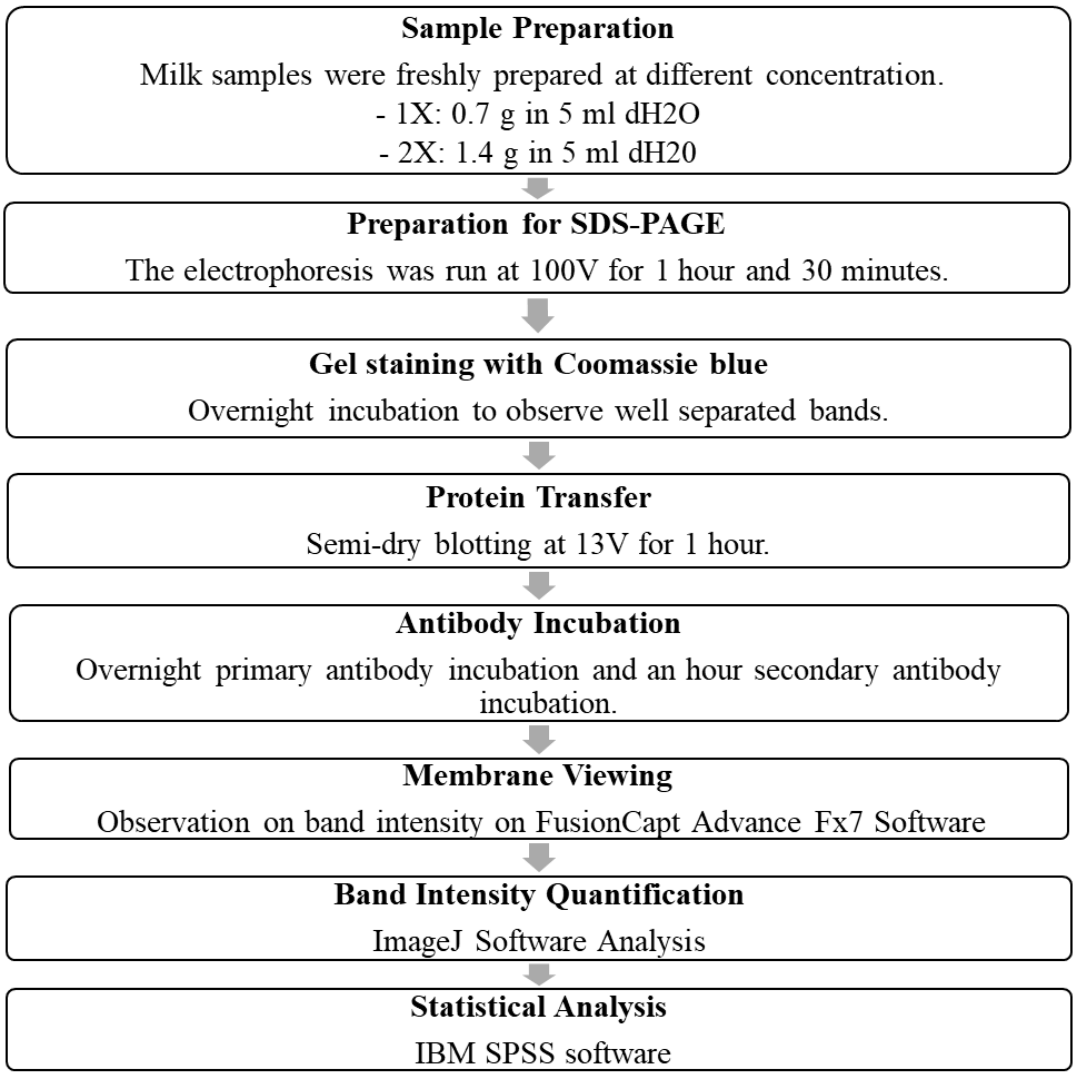

Figure 1.1 Flowchart of the determination of chimeric secretory IgA: TB epitopes in goat milk.

CHAPTER 2

LITERATURE REVIEW

2.1 Tuberculosis: types, causes, diagnosis and treatment

Tuberculosis is recognised as a significant global health issue, ranking as the ninth most prevalent cause of mortality worldwide prior to the emergence of the COVID-19 pandemic (Houda Ben *et al*., 2018). The worldwide health care system has seen severe repercussions because of the COVID-19 pandemic. Consequently, there has been a substantial decrease in the number of new TB cases in 2020, which has persisted since early 2021. This decline has led to disruptions in the provision of TB treatment services (Jeremiah *et al*., 2022). Therefore, the persistence of TB is observed, particularly in countries with a high burden of the disease and poor wealth, mostly due to inadequate access and several contributing variables that perpetuate the ongoing mortality associated with TB. Tuberculosis can be characterised as a clinical illness resulting from infection with Mtb, which has the potential to impact various essential organs as a multi-systemic infectious disease.

2.1.1 Types of tuberculosis

There are two distinct forms of tuberculosis infections: active tuberculosis and latent tuberculosis. Active tuberculosis refers to the condition in which an individual harbours the tuberculosis organism and has symptomatic manifestations, thereby enabling the transmission of the illness to others. In cases of latent tuberculosis, individuals harbour Mtb without presenting any clinical manifestations, potentially

attributable to the immune response effectively combating the infection and exerting a suppressive effect. Individuals with latent tuberculosis infections do not pose a risk of transmitting the disease to others. However, it is possible for the bacteria to undergo reactivation at a later stage when the individual's immune system becomes weakened over time, leading to the manifestation of an active tuberculosis infection. The investigation of mucosal vaccination against tuberculosis has garnered significant attention in the field of research. This area of study aims to address both active tuberculosis and latent tuberculosis, which has the potential to reactivate into active tuberculosis at a later stage. Tuberculosis can also be classified according to the specific organs it infects. When Mtb has an affinity for the lungs, it gives rise to a medical illness known as pulmonary tuberculosis (PTB). Conversely, when Mtb affects organs other than the lungs, it can lead to the development of a condition referred to as extrapulmonary tuberculosis (EPTB). Based on the findings of the European Centre for Disease Prevention and Control (2022), it shows that a cumulative count of 33,148 instances of TB was documented during the specified period. Among these cases, 73.1% are identified as PTB, while 21.5% are classified as EPTB. The remaining cases were documented as instances of concurrent pulmonary-extrapulmonary tuberculosis or as cases where the tuberculosis location was not specified.

2.1.1.1 Pulmonary tuberculosis

Pulmonary tuberculosis is widely recognised as the predominant clinical presentation of the disease, accounting for around 85% of reported tuberculosis cases on a global scale (Rolo *et al*., 2023). The symptoms associated with PTB include the

expectoration of phlegm and blood, persistent fever, nocturnal perspiration, and thoracic discomfort.

2.1.1.2 Extrapulmonary tuberculosis

Once Mtb infects any part of the body other than the lungs, it is referred to as extrapulmonary TB. The most common anatomic sites for EPTB are lymph nodes and the pleura, although it can be found almost anywhere in the body (Baykan *et al.*, 2022). Among other types of tuberculosis infecting other organs are pleural tuberculosis that affects the lining of the lung, skeletal tuberculosis due to infection in the spinal column, brain tuberculosis, bladder and kidney tuberculosis, joint tuberculosis, gastrointestinal tract tuberculosis, and miliary tuberculosis (Toshi, 2021).

2.1.2 Causes of tuberculosis

Mtb, a bacterium known for its rod-shaped morphology, is responsible for the primary etiology of TB. Mtb exhibits a strict reliance on oxygen for its proliferation and thrives in environments abundant in both oxygen and nutrients. The growth rate of this organism is characterised by a slow pace, and the manifestation of symptoms may require a considerable amount of time. The bacterium possesses a protective membrane that effectively shields it from the host body's natural immune response, hence impeding the immune system's ability to combat and eliminate the bacteria (Toshi, 2021). Therefore, it is evident that Mtb can disseminate within the human body via the circulatory and lymphatic systems. Upon affecting a specific region, it subsequently gives rise to the development of a pathological structure known as a granuloma, which serves as a diagnostic indicator for medical professionals to ascertain the presence of Mtb infection in the patient. The formation of a fibrous

capsule around the previous granuloma can serve as a protective barrier for Mtb in cases of latent tuberculosis (Toshi, 2021). This latent infection can later transition into an active form when the immune system becomes compromised.

2.1.3 Diagnosis of tuberculosis

The diagnosis of TB can present challenges, particularly when patients are asymptomatic and in a latent state. Therefore, the initial diagnostic procedure will involve obtaining a comprehensive clinical history to ascertain the presence of clinical indicators suggestive of tuberculosis. A sputum examination is conducted on people exhibiting symptoms indicative of pulmonary tuberculosis. Nevertheless, patients harbouring latent tuberculosis infections do not exhibit the presence of Mtb in their sputum (Toshi, 2021).

Several diagnostic procedures will be conducted to confirm the diagnosis, including the Mantoux test, which entails the subcutaneous injection of inactivated Mtb bacteria. The administration of this injection is associated with the occurrence of an allergic reaction, which manifests as localised swelling, erythema, and induration at the site of injection. These reactions indicate a positive diagnosis of TB. Nevertheless, there is a potential for the TB test to yield a positive result after an individual receives the BCG vaccination, hence diminishing its diagnostic accuracy (Toshi, 2021). Additionally, a chest radiograph can be employed as a diagnostic tool for pulmonary tuberculosis, wherein the presence of patches in the upper lung region may be observed. Patients diagnosed with miliary tuberculosis will exhibit numerous dispersed lesions throughout the pulmonary fields, indicating a more extensive dissemination of the infection (Toshi, 2021).

In addition, the use of ultrasound imaging of the abdomen serves as a diagnostic modality for identifying potential infections or abnormalities within this anatomical region. Notably, alterations in the liver and other essential organs can be visualised when they are affected by Mtb infection (Toshi, 2021). The diagnosis of Mtb infection in the bladder can be achieved using a urine test, wherein the presence of pus cells in the urine is indicative of the illness. However, after the urine culture is conducted, Mtb cannot be identified, resulting in a condition known as sterile pyuria. This is considered one of the distinguishing features of tuberculosis infection in the bladder (Toshi, 2021). Finally, the detection of Mtb infection in the brain can be accomplished by employing a computed tomography scan of the cranial region, which enables the assessment of the degree to which the brain is impacted (Toshi, 2021).

2.1.4 Treatment of tuberculosis

Obtaining appropriate therapy for TB, even in the absence of symptoms as observed in latent tuberculosis, is of utmost importance. In the case of pulmonary tuberculosis, the administration of standard medications, including isoniazid, pyrazinamide, ethambutol, and rifampicin, is recommended for a duration of at least six months to achieve optimal therapeutic outcomes. Verver *et al*. (2005) reported that the concurrent administration of these four medications results in a fast bactericidal effect within the initial two-month period. Nevertheless, it is important to note that relapses and the emergence of multi-drug resistant strains are common outcomes when the therapy is extended for an additional 4 months to eradicate the remaining bacilli (Zhang, 2004).

Directly observed therapy is a method employed to assure the comprehensive completion of treatment. Noncompliance with medication regimens for PTB can result in the development of drug resistance, leading to the emergence of multi-drug resistance tuberculosis (MDR-TB) (Carey, 2018). MDR-TB is a medical illness characterised by its resistance to the standard antibiotics employed in its treatment, namely isoniazid and rifampicin. This resistance can be attributed to factors such as the administration of improper drugs for tuberculosis treatment, premature discontinuation of treatment, and the utilisation of pharmaceuticals of suboptimal quality (Carey, 2018). According to the WHO, the primary factor contributing to the emergence of MDR-TB can be attributed to the inappropriate prescription of drugs. However, it is conceivable for people who have not been adhering to TB medication regimens to develop drug-resistant strains of the disease. The treatment duration for MDR-TB might extend up to a period of two years, which is accompanied by significant financial costs. In alternative scenarios, it is also conceivable for the condition of extensively drug-resistant TB to manifest, necessitating a regimen of highly comprehensive medicine (Carey, 2018).

An additional approach to mitigating and managing the mortality associated with TB disease is the utilisation of vaccination. Presently, there is a significant body of research being conducted by scholars to formulate a vaccine that may effectively target both active and latent TB. This is necessary due to the limitations of the present BCG immunization, which exhibits suboptimal efficacy and is influenced by various other factors.

2.2 Bacillus Calmette-Guerin (BCG) vaccination

Inactivated bacterial toxins or whole, dead microorganisms are used to develop one type of vaccine (Oyston and Robinson, 2012). Historically, whole microorganisms with negligible pathogenicity have been used in vaccine research (Plotkin, 2009). Between 1921 and 1927, researchers tested the efficacy of the BCG vaccine (Andersen and Doherty, 2005). Clinical trials of the BCG vaccine for tuberculosis prevention in children have shown impressive results, especially in the prevention of TB meningitis. The global dissemination of BCG followed the extension of the World Health Organisation's tuberculosis immunisation programme to countries outside of Europe's endemic areas. The effectiveness of this vaccination in preventing pulmonary tuberculosis in adults ranges from 0% to 85% (Fine, 1995), making it unpredictable and unreliable. Even though more than 100 million newborns worldwide receive the BCG vaccine every year, there is persistent controversy over the efficacy of the vaccine in preventing and lowering deaths from tuberculosis.

The BCG vaccine has been administered for a century and is considered one of the most extensively utilised vaccines on a global scale (Lange *et al*., 2021). The vaccine in question is renowned for possessing a thoroughly established safety profile and exhibiting a high degree of cost-effectiveness. Nevertheless, the vaccine has garnered controversy considering its effects on older children and adults (Fine, 2001). There are still unresolved inquiries pertaining to the BCG vaccine and its implications for the tuberculosis epidemic. Moreover, the efficacy of the vaccine in providing protection against pulmonary tuberculosis remains uncertain due to its unpredictability.

Several factors have been identified as contributing to this variation, including the sensitization caused by environmental mycobacteria, the timing of vaccination administration, and the rigorous tuberculosis testing (Mangtani *et al*., 2013). A study conducted by Biering-Srensen *et al*. (2017) suggests that the efficacy of BCG vaccination may be influenced by sex-specific variations. One of the most contentious elements pertaining to the BCG vaccination revolves around the extent of its efficacy in providing long-term protection against latent tuberculosis and its potential for preventing reactivation. Thus, the development of a novel vaccine that focuses on both active and latent TB has become an interest among researchers to fill the gap in current BCG vaccination.

2.3 Induction of mucosal immune responses against tuberculosis

The organism that causes tuberculosis, Mtb may spread through the air and is highly contagious from person to person (Smith, 2003). The infection is often detected in the lungs. Once the infection has spread to the lung parenchyma, as shown in Figure 2.1, dendritic cells and local macrophages try to devour the bacteria to transport them to the draining lymph nodes. The infection has effectively spread into the lung parenchyma. T-lymphocytes, the most significant immune system mediators during an infection, will then be stimulated. As a result of this process, massive granulomas will grow in the lung parenchyma, continuing to harbour Mtb.

Granulomas are a sign of tuberculosis that can be seen in people who are infected with the disease. When the Mtb reaches this stage, it enters a latent state, halting all metabolic and replicative activities. When tuberculosis kills out many macrophages, granulomas can become caseous or necrotic. This happens once the granulomas'

integrity has been damaged. The Mtb returns to its normal metabolic state after the replication process is completed. When granulomas burst or become caseous, active infections can spread, and the illness can spread even further when infected cells are expectorated out of the body. Granulomas are an extremely common cause of respiratory infections. Once established in the lungs, the infection can "seed" in other regions of the body or spread within the lungs themselves, resulting in diseases such as pulmonary tuberculosis. A secondary tuberculosis infection can appear anywhere between a few months and a few decades after the first tuberculosis infection has cleared up. According to Cooper (2009), people who are immunocompromised, such as those with HIV or who have recently received an organ transplant, are more likely to get this secondary infection. Factors such as malnutrition, diabetes, and tobacco use raise the likelihood of developing this secondary disease. In contrast to the reactivation of a local infection, this reactivation can also be initiated by a first tuberculosis encounter (Andersen and Doherty, 2005).

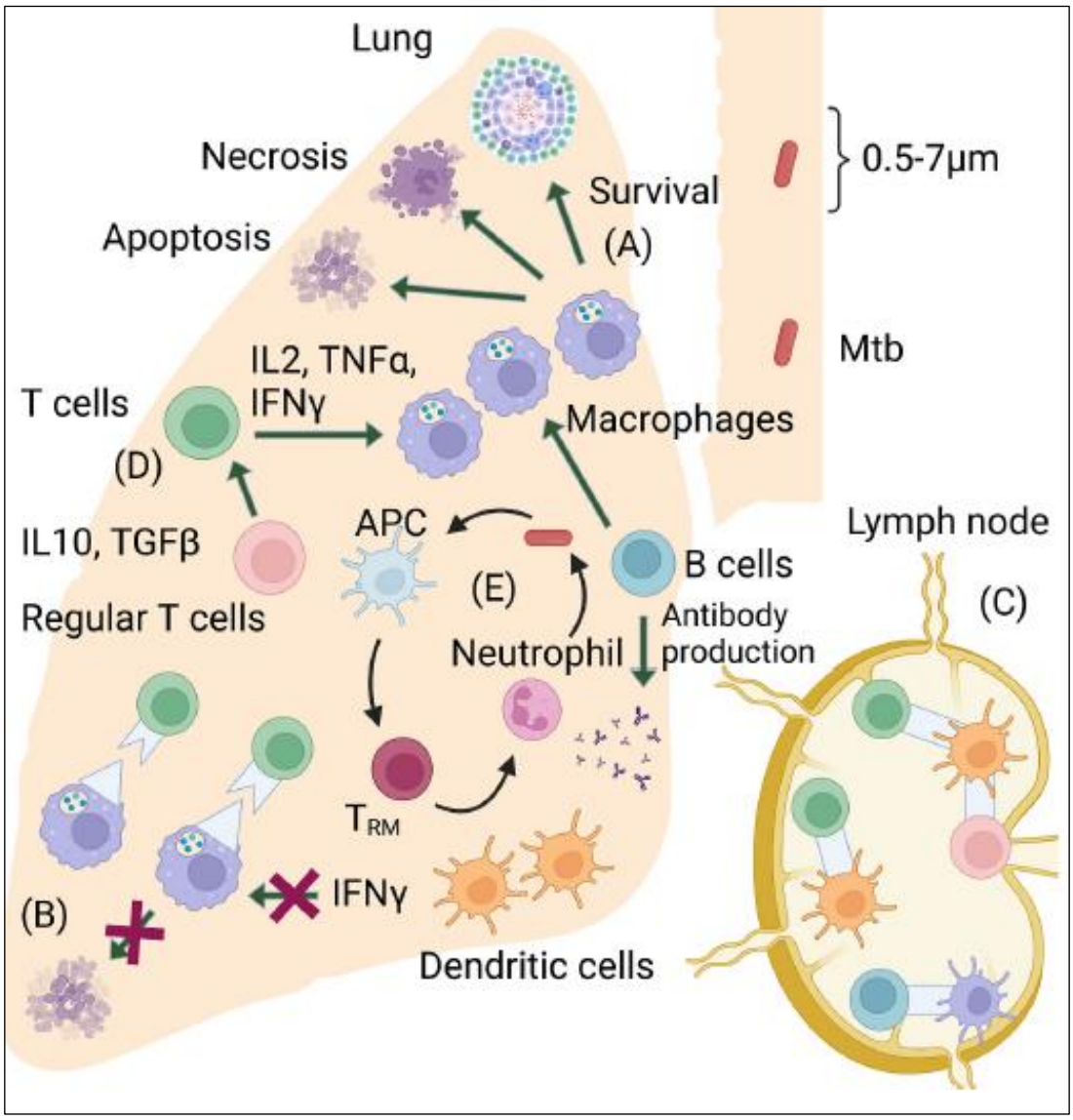

Figure 2.1 Illustration of antimycobacterial immune mechanisms in the lung and lymph nodes by Duong *et al*., (2023). (A) described the process that occurs after the inhalation of Mtb travelling down the lung; (B) described the inhibition of phagolysosome formation; (C) described the function of dendritic cells; (D) showed the activity of B cells and T cells in controlling bacteria; and (E) showed how bacterial clearance is done upon the release of cytokines.

Figure 2.1 above is a diagram of how the immune system fights mycobacteria in the lungs and lymph nodes. The inhaled Mtb is the first to enter the lung, and the macrophages and dendritic cells nearby that can detect these germs follow it. In scenario A, the macrophages undergo apoptosis or necrosis, and the dead macrophages then release the germs into the lungs. This pathogen continues to spread. Granulomas are formed when surviving macrophages gather to form a cluster. This cluster is known as a granuloma. If these granulomas are unable to degrade on their own, they will continue to cluster together inside the cell, which will result in a condition of dormancy.

B shows the pathogen, Mtb, which can stop the formation of phagolysosomes and apoptosis to avoid the host's antibacterial protection system. This bacterium can also stop macrophages from responding to IFN- γ. In C, it can be seen the journeys of dendritic cells from the lung to the lymph nodes for presenting the Mtb antigen to naive T-cells, B cells, as well as regulatory T-cells before activating them. In D, cytokines and antibodies are made by T cells and B cells that have been activated properly. These are then used to stop the growth of bacteria. Sáenz *et al*. (2013) found that regulatory T cells can also stop inflammation by making IL-10 and TGF-β. In E, when Mtb encounters APCs, they release cytokines like IL-12, which then activate memory cells in the lungs (T_{RM}), which then release IFN-γ, which helps recruit more neutrophils to get rid of the bacteria that cause tuberculosis (Ge *et al*., 2019).

2.4 CD4-T cells induction by Th17

According to Cooper (2009), CD4-T cells, and more specifically IFN- γ and TNF, are essential effector cytokines that control this pathogen. Most of the new

tuberculosis vaccines that are now under investigation are administered by parenteral route. Mucosal administration, on the other hand, has been proven to be effective at boosting pulmonary immunity and acting as a preventative measure in animal models of the disease (Orr *et al*., 2015). According to the findings of a study that examined the immunogenicity of parenteral and mucosal vaccination (Orr *et al*., 2015), mucosal vaccination, such as intranasal immunisation, triggered CD4-T cell responses of much greater quality than parenteral vaccination. This is the conclusion drawn from a comparison of the two types of vaccination. The parenteral vaccination induced a conventional Th1 immune response, which is distinguishable by IFN-generating specific CD4-T cells in the spleen and lung (Orr *et al.*, 2015). The mucosal immunisation, on the other hand, transitioned to a Th17 response by creating IL-17A by CD4-T cells (Orr *et al*., 2015). According to findings published by Chen *et al.* (2011), Th17 cells play a crucial role in the process of building protective immunity against a wide variety of bacterial illnesses. Hernandez-Santos *et al*. (2012) conducted research that found that the cytokines produced by the Th17 lineage, IL-17A, IL-17F, and IL-22, have a significant impact on the host's immunological response to bacterial infection.

Inhibiting IL-17's downstream signalling pathways has been shown to increase pathogen load and mortality in mouse models of the disease (Wüthrich *et al.*, 2011), and vaccination of mice results in significant Th17 responses in the lungs. To get rid of the pathogens that set off the immune response, it is necessary to prevent the antibody from switching classes (Mitsdoerffer *et al.*, 2010). According to research by Rangel-Moreno *et al.* (2011), IL-17 is the driving force behind the formation of inducible, lymphotoxin-independent, bronchus-associated lymphoid tissue in the lung. Th-17 cells can promote B lymphocyte entrance into the lung by boosting pIgR

expression on bronchial epithelial cells (Jaffar *et al*., 2009). The amount of IgA and IgM that enters the airway lumen is regulated by this pIgR. Czerkinsky and Holmgren (2012) claimed that sIgA antibodies were produced in response to oral vaccination, specifically in the mammary gland, the stomach, and the small intestine. As they prevent Mtb from adhering to and penetrating epithelial cells in the respiratory system, sIgA antibodies are essential for mucosal immunity. Czerkinsky and Holmgren (2012) found that sIgA antibodies contribute to the removal of infected cells and antigens from the mucosa.

Enhanced CD4-T cell responses in the spleen revealed that intramuscular immunisation caused systemic CD4-T cells, in contrast to intranasal vaccination, which generated tissue-resident CD4-T cells. Another piece of data suggesting that intranasal immunisation selectively boosts tissue-resident memory cells is that CD69 expression is higher in lung-resident cells induced by intranasal vaccination than in cells stimulated by intramuscular immunisation. Th17 cells, also known as effector memory cells, are known to be long-term residents of mucosal tissue (Kryczek *et al.,* 2011). Th17 cells are versatile in that they can be reprogrammed into either Th1 or Th2 lineages in response to the cytokine milieu seen in mucosal locations. Th17 cells, for instance, can be reprogrammed into IFN-producing Th1 cells by IFN or IL-12 (Lee *et al.,* 2009). Despite the fragility of Th17 cells in inflammatory conditions, vaccination increases stable, long-lived memory Th17 cells in the absence of inflammation (Lindenstrm *et al.,* 2012). Therefore, vaccination of mucosal surfaces like the lung, the source of infection, is a manageable strategy to produce long-lasting immune responses.

2.5 Structural and functional of immunoglobulin A

According to genetic comparisons and functional analyses, immunoglobulin A, also known as IgA, is present in all mammals and animals. Two Cα genes, IgA1 and IgA2, have been identified in humans as encoding for distinct IgA subclasses (Kawamura *et al.*, 1992). IgA molecules are known to consist of a pair of heavy chains and a pair of light chains that are identical to one another. 90% of human IgA is IgA1, while only 10% is IgA2 (Woof and Russell, 2011). Therefore, IgA1 can be considered the more common of its two subclasses.

2.5.1.1 Basic monomer unit of immunoglobulin A

In Figure 2.2, the fundamental structure of IgA includes two subclasses, IgA1 and IgA2, both with their own set of effector mechanisms mediated by the Fc region, which is connected to two identical Fab regions via a hinge region. IgA's fundamental monomer unit is stabilised by inter-chain disulfide bridges, which allow the monomers to pack closely together and bind to one another. IgA's basic monomer unit is made up of four globular domains (VH, C1, C2, and C3) in the heavy chain and two globular domains (VL and CL) in each light chain as can be seen in Figure 2.2. Woof and Russell (2011) revealed how the configuration of IgA1 Fc complexes may vary depending on the ligands present. No disulfide bonds are present between the heavy and light chains in IgA2. Instead, the light chains are connected by disulfide bridges.

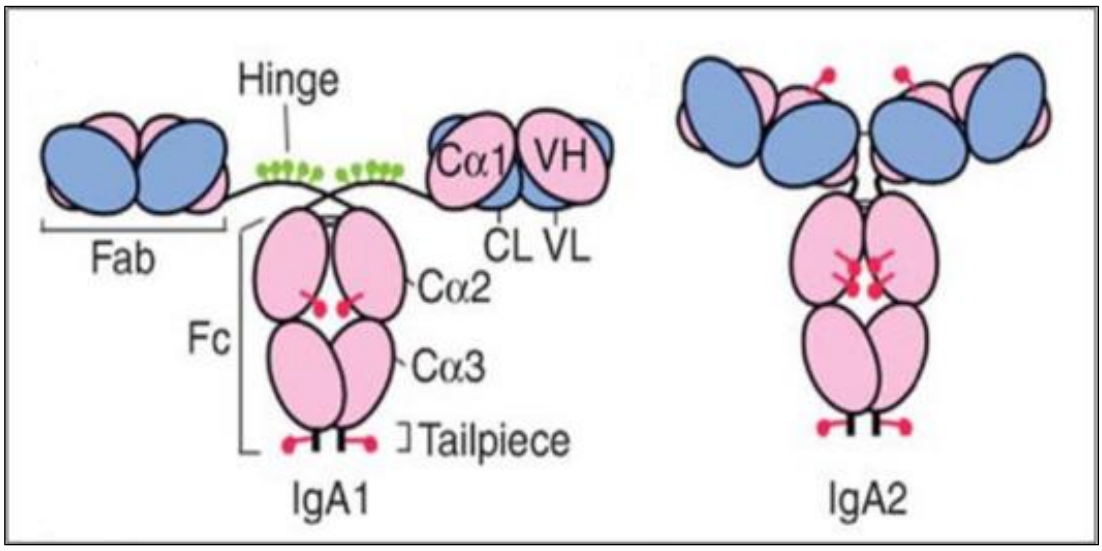

Figure 2.2 The structure of human IgA by Woof and Russell (2011).