

Genetic and Genomic Landscape of Tuberculosis

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Human Tuberculosis (**TB**) is an infectious disease mainly caused by the *Mycobacterium tuberculosis* (**Mtb**) Complex. TB is an airborne disease, since the Mtb travels to the lungs via droplets from the atmosphere. In the lungs, the Mtb is phagocytosed by macrophages, where it can survive long periods. Classic symptoms of pulmonary TB are chronic cough, bloody sputum, fever and weight loss. Non-pulmonary TB could cause many other kind of symptoms depending on the harboring organ.

The tuberculosis disease has been present in mankind for over four thousand years. It has been identified in human skeletons in Europe and Middle East. During XVIII and XIX century, it reached epidemic proportions; however, it was until the XX century, that the mortality rate associated to TB began to decrease. The main reason for the mortality was due to a vaccine development and massive vaccination programs that used the *Calmette-Guérin bacillus* (**BCG**) strain and antibiotics as streptomycin, rifampicin and isoniazid to control the epidemics. However, this negative slope ended with co-infections as the Human Immunodeficiency Virus (**HIV**) and socio-economic

factors as poverty. Also, Multidrug Resistant (**MDR**) strains begin to appear during this century due to the wrong use of antibiotics. MDR strains are those mycobacteria that resist to rifampicin and isoniazid at least. MDR-TB has been defined by the World Health Organization as the major world challenge to combat TB [1].

TB in the actuality is still a worldwide disease that causes 1.5 million of deaths a year and it is considered that around one third of the global population is infected with Mtb without developing the disease, condition called latent TB. HIV infection is one of the main risk factors for the activation of the mycobacteria in latent cases. In 2013, WHO considered that 9 million people developed TB, from which 360 thousand were HIV co-infected and 510 thousand was in infant population (below 15 years old) [2].

Around 90% of people infected with Mtb will not develop TB, which could be due to the total elimination of the bacteria or that the immune system has managed to avoid its development and the Mtb remains in a latent state inside macrophages. Although we still do not know the molecular reason of this behavior, several factors need to be considered. These factors are the strain involved, because different strains indicate different virulence level, a possible genetic susceptibility to the disease and environmental factors involved in the health of the immune system as poverty or nutrition. The genetic factor is given by a delicate balance of the expression of the genes involved with the efficiency and effectiveness of the immune response, being that in the presence of functional DNA polymorphisms and/or mutations (in coding or regulatory regions), the immune response can be insufficient or inadequate. In the particular case of TB, if a gene directly involved in the defense against this bacillus is affected by a functional variation, then we could say that the host is susceptible to TB [3].

At the present moment, we have not been able to decode in detail the immune response of an Mtb infection. However, by murine model experiment, we know that T_H1 cells as well as the cytokines Interleukin (**IL**)-12, Interferon (**IFN**)- γ and the Tumor Necrosis Factor (**TNF**) are essential components to Mtb control [4-6]. However, the molecular mechanism of the activation of the disease is still unknown [5].

INFECTION PROCESS

During exposure, when the mycobacteria enter the host by airways, it interacts with the alveolar epithelium and with dendritic cells via some membrane proteins called Toll Like Receptors (**TLRs**), which activate the immune response. This provokes that the dendritic cells phagocytose the pathogen and migrates to a lymph nodes to present the antigens to T Lymphocytes (**TL**) activating the adaptive immune response. Promptly, TL migrates to the region of inflammation along with macrophages to try to control the mycobacteria invasion. The Mtb is recognized by these immune cells via TLRs to phagocytose the pathogen (innate immune response) or create an adaptive immune response by macrophages or TL respectively [7] (Figure 1).

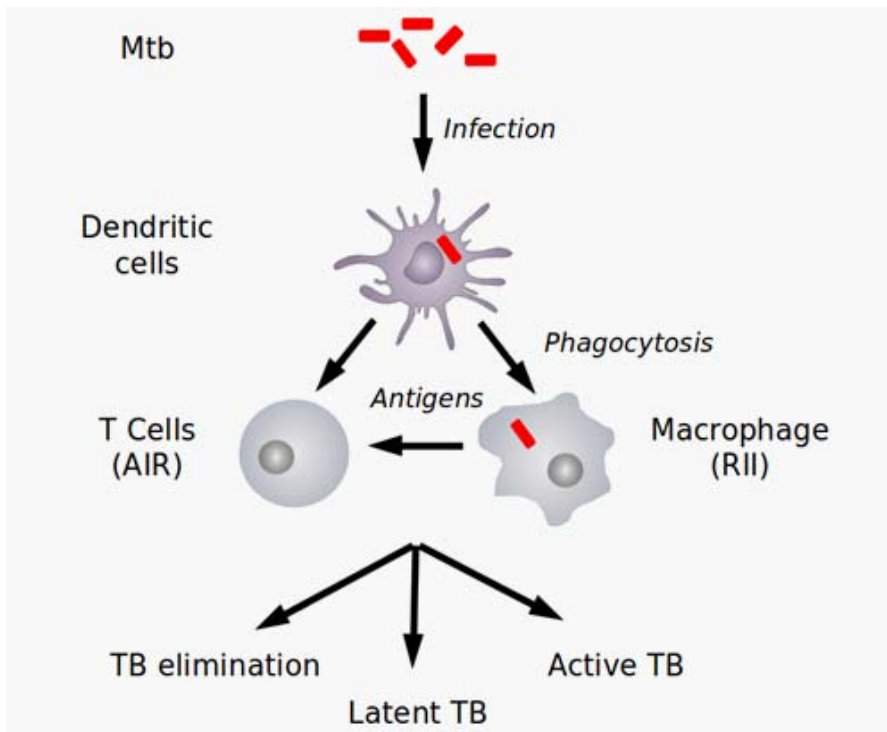


Figure 1: Diagram of the general response of immune response to *M. tuberculosis*.

The dendritic cell phagocyte the mycobacteria using several cytokines to produce the IIR, which calls macrophages to phagocyte and eliminate the invading pathogen. Besides, dendritic cells also activate the AIR by presenting antigens to T cells. Three possible outcomes might occur, the total elimination of the Mtb, the persistence of Mtb inside the macrophages but in an inactive or latent form and the activation of the disease. Image: Flores Saiffe Farias Adolfo.

The activation of TLRs in the membrane of the macrophages and dendritic cells trigger the production of chemokines and cytokines that produce inflammation. Once activated T cells return to the inflammation site, they enhance the formation of granulomatous lesions made of macrophages, B cells, dendritic cells and T cells. The granuloma can cause local necrosis that can be extended to the alveolus. Inside the parenchyma and inside the granuloma, fibrosis is formed with a tubercle shape [1,8].

***M. TUBERCULOSIS* LATENCY**

When the Mtb lodge in the pulmonary epithelium inside macrophages forming infectious granulomas, it can remain in a latent form for years without causing disease; this state is called latent TB. However, the decrease in the host capacity of retaining the Mtb can cause infection (active TB) and unleash the TB symptoms. It is possible that the bacteria in the active state migrates to other organs as the lymph nodules, bones, brain or others and cause extra pulmonary TB [5].

Lots of research has been made in the last years trying to understand the molecular mechanism that makes possible the latency of the Mtb inside the macrophages. It is known that at least three mechanisms are involved: (1) the Mtb avoids the maturation of the phagolysosome, (2) the Mtb diminishes the production of nitrogenous reagents and (3) the Mtb controls the antigen presentation to avoid the detection of the infected macrophages by the CD4⁺ TL [1,9-11]. The first mechanism has been the most significant in explaining the persistence of Mtb inside macrophages [12]. This process is known as the phagolysosome maturation arrest by Mtb, in which the fusion of the phagosome with the lysosome is avoided. Also, Mtb creates a favorable environment inside the phagosome to its persistence, obtaining energy from lipids [13].

Cytokines as the TNF- α , IL-1 and IFN- γ that are produced by TL during a bacterial infection can induce the production of reactive nitrogen species as Nitric Oxide (**NO**) in macrophages via Nitric Oxide Synthase 2 (**NOS2**). These reagents can modify or break bacterial DNA inhibiting its growth or interact with accessory proteins creating malfunctions [14,15]. However, Mtb decrease the production of reactive nitrogen species by inhibiting the pathway of the NOS2 activation. Studies have proven that Mtb has genes associated to the resistance against reactive nitrogen intermediates. Evidence keeps growing that the reactive nitrogen intermediates have a role in the defense against Mtb, however, they are considered controversial [9,16,17].

TL CD4⁺ recognition of infected macrophages depends on the antigen presentation of the Major Histocompatibility Complex II (**MHCII**), regulated by IFN- γ . However, Mtb in infected macrophages, inhibit the induction of MHCII presentation to TLs via IFN- γ [18]. Several works have found some intermediary molecules (as TLRs and IFNs) involved in this inefficient antigen presentation [10,18-21].

INNATE IMMUNITY AGAINST *M. TUBERCULOSIS*

The human defense system depends in the first instance in the activation and integrity of the innate immune response. It is the first line of defense of the human, and works through several tissues as epithelium, cell types as lymphocytes and molecules, as the epithelium and potential destructive cells as the Natural Killer (**NK**) cells and different polypeptides, cytokines and other effector molecules.

The main function of the epithelial barrier is to isolate the organism from potentially harmful molecules or microorganisms in the environment. If a pathogenic organism passes through the skin, it could be recognized by membrane receptors in the phagocytes, which could unleash the production of cytokines such as the TNF, interleukins, chemokines and others. These cytokines has chemotactic functions that activate and call metabolites and other cells to the infection zone in a process called inflammation. These cells could be leukocytes, NK lymphocytes among other cells of the adaptive immune response. The leukocytes called are mainly neutrophils and monocytes. The latter are the precursors of macrophages.

As a part of the innate immune response, macrophages and neutrophils can engulf (phagocyte) a pathogen to the cytosol forming a phagosome. This occurs once the phagocyte is activated by its exposure to TNF- α , IFN- γ or some agonists of the TLRs [10]. Once the pathogen is engulfed into a phagosome, these can integrate lysosomes forming a structure called phagolysosome in a process highly regulated and complex. This fusion between the phagosome and the lysosome has the objective of provoking lysis of the pathogenic cell by intermediary proteolytic and lipolytic enzymes as well as the huge amount of free radicals acidifying the environment, killing or harming the pathogen [10,22].

NK lymphocytes can kill virus or bacteria infected cells that are intracellular (into a phagosome) and cancer cells. In difference of the adaptive immune response, NK cells act spontaneously against any target, recognizing changes in the cell envelope. They are activated after recognizing a glycolipid presented by the major histocompatibility complex class I.

The Antimicrobial Peptides (**APs**) are molecules produced by the innate immune response and are known for their activity against pathogenic microorganisms (like bacteria, fungus and some viruses with a capsid) and the regulation of the immune response. In mammals there are two kinds of APs, the cathelicidins (forming alpha helices) and the defensins. Some of them directly attack the pathogen's cell membrane by depolarizing it or inhibiting protein, DNA or RNA synthesis, however their specific molecular pathways are not yet well described [23] despite several models have been proposed.

One of the most studied AP in humans is the cathelicidin LL-37. It is a cationic 18-kDa peptide. It has been found to be expressed in several epithelial cells (e.g. lung epithelium), monocytes, neutrophils, B cells among others. LL-37 regulates some functions in macrophages and induces the expression of cytokines and other relevant proteins of the immune response. It has an antimicrobial activity against bacteria, fungus and parasites and enhances the migration of T lymphocytes to the site of the infection. The cathelicidin LL-37 sequence information comes from a single gene called *CAMP*. The transcription of *CAMP* can be regulated by the presence of 1,25 Dihydroxyvitamin D₃ (**1,25D₃**) in a Vitamin D Receptor (**VDR**) dependent manner.

In the case of the presence of Mtb, in monocytes the *CAMP* gene is activated via TLR2/1 in a vitamin D dependent way. It is known by an experiment in monocyte cells, that when they are exposed to Mtb, 1,25D₃ activate the immune response against Mtb [24] by enhancing the maturation of the phagolysosome [25]. Therefore, a vitamin D deficiency, or a deficiency in the signaling pathway to activate the immune response, could lead to a susceptibility to TB. LL-37 combats the Mtb by enhancing phagocyte formation and its maturation into phagolysosome [3].

Defensins are cationic polypeptides mainly produced by lymphocytes, neutrophils and some epithelial cells. They have shown microbicide, cytotoxic and immunomodulatory activity. They are classified as alpha-defensins, beta-defensins and theta-defensins according to their molecular scaffold shape, cysteine residuals and disulfide bonding quantity.

There have been identified six different genes those codes for defensins. One of them, the Beta-Defensin 1 Gene (**DEFB1**) has been observed expressed in prostatic cells, renal cells and urogenital epithelial cells. The expression of this gene produces a polypeptide called Human Beta Defensin 1 (**hBD-1**), and it can inhibit the proliferation of some cancer cells including in bladder and kidney [26,27]. It also induces the immune response by enhancing the migration of immature dendritic cells and T cells.

It has been demonstrated that *M. bovis* BCG cellular envelope components stimulate the transcription of the *DEFB1* gen in endothelial cells and lung epithelium via the transcription factors C/EBP β , AP-1 (JUN) and CP2 after the stimulation with the bacteria [28]. However the mechanism by which the defensin combats the infection is unknown.

GENETIC LANDSCAPE OF IMMUNITY

The efficacy of the immune system depends of a very complex interaction network between sensing the pathogen and activating the defense mechanisms. This interaction network has several hundred or probably thousands of different proteins interacting in the correct place of the immune system (specific cells and organs) and with enough speed to avoid the rapid growth of the pathogen.

Every protein of the immune system that is not constitutively expressed, is synthesized according to a robust gene expression control that involves several factors: (1) enough concentration of specific transcription activator proteins, also known as transcription factors, (2) an accessible DNA sequence in the promoter region that is specific for the transcription factors to recognize it and interact with it to enhance the transcription of the protein and (3) a delicate balance of co-interactions between activators and repressors in the promoter of a target gene. We must understand that each of these transcription regulator proteins comes from its own process of transcription regulation. This could help us understand the complexity of the immune system signaling pathways and the genetic landscape involved in the transcription of the proteins involved in this system.

Lot of resources are invested in research to advance in the comprehension of these complex interactions and in the genetics involved in it. One of the main reasons is that the DNA variations between different populations can have an impact on the efficiency of the immune system. A DNA variant is called polymorphism or mutation depending on its demographic frequency and these variations can occur in critical DNA regions for the immune system. For example, if a variant is within a gene, it could modify the structure of the protein, or if the variant is within an expression regulatory region (e.g. transcription factor binding sites), the concentration of certain protein of a signaling pathway, could be altered, and thus, the defense mechanisms compromised.

TUBERCULOSIS RELATED GENES

Recent technology has provided the tools to analyze these systems from different perspectives, including variomics and transcriptomics. From the variomics perspective, it is possible to identify DNA variations in the host that are significant in a case-control experiment. These types of associations (variant – disease) are also called Genetic Association Studies or Genome Wide Association Studies (**GWAS**) when they are performed in the whole genome. Interestingly, several genetic association studies performed in tuberculosis patients vs. controls have identified DNA variations in regions related to the immune system, suggesting that some genetic variants could promote a genetic susceptibility to the activation of latent TB. These DNA regions are sometimes, within genes (exons or introns) or could be in the promoter region of genes part of the immune system such as IFN- γ [29], IL-22 [30], LL-37 [31], hBD-1 [3] as many others [32-35].

IFN- γ is a cytokine closely involved in the innate and adaptive immune responses in the defense against viral and bacterial infections. Some alleles of this gene have been associated to a major susceptibility to TB in case-control studies in South African population [36]. Other studies have found protective alleles in this gene by using a meta-analysis of eleven association studies [37]. Something similar has been found in interferon receptor genes that have a crucial role in the activation of IFN pathways [38,39]. Other study found susceptibility alleles in the promoter of *IFNGR1* [40].

The TNF is a multi-functional cytokine that promotes inflammation and is secreted by monocytes mainly. It has been observed closely related to IFN- γ . It has been observed that inhibition of TNF resulted in a major susceptibility to TB in mice [41]. Other studies strongly suggested that there is a strong genetic influence on TNF expression in TB [42]. Several other studies have associated polymorphic regions within this gene and in its promoter region [43,44]. Other studies have also found susceptibility alleles in the TNF receptor gene [42].

Interleukins (**ILs**) are a big family of cytokines that were first observed in leukocytes. They are tightly involved in the innate and adaptive immune system and are synthesized mainly in CD4⁺ T lymphocytes, monocytes/macrophages, and endothelial cells. Association studies have been able to associate SNPs from these *loci* to TB susceptibility, for example, within the IL-1 β promoter and exon [45], IL-6 in promoter [46], IL-8 in exon and promoter [47] and IL-10 in the promoter [48].

TLRs are a family of membrane proteins that are part of the innate immune system. Several agonists can interact with these proteins, including conserved microbial molecules. Once a TLR is activated, it unleashes the immune response by a complex waterfall of molecular interactions. They are expressed mainly in monocytes/macrophages, dendritic cells and B lymphocytes, however other cell lines could express some types of TLRs. There are many genes coding for TLRs, however only a few of them have been associated to the susceptibility to TB. Some non-synonymous mutations in the coding region of TLR1 [49], TLR2 [50], TLR4 [51], TLR8 [52], and

TLR9 [53] and one mutation on the promoter of TLR2 [53] were associated to an increase on TB disease risk.

Vitamin D has also been associated to TB prevention by activating the innate immune response and therefore the Vitamin D Receptor (**VDR**). The VDR is an intracellular hormone receptor that specifically binds 1, 25-dihydroxy vitamin D3 and mediates its effects. It has been proven that the downstream pathway of VDR activation involves the transcription of APs as LL-37 [54]. Many genetic association studies have been performed in this gene in association with TB finding several susceptible alleles (review [55]).

The transcription of some APs has been observed variable in different populations, which could mean that it has an important genetic factor. This means that there might be genetic mutations or polymorphisms that could be modifying the signaling waterfall to the activation of the transcription of these APs. For example, there are some Single Nucleotide Polymorphisms (**SNP**) in the promoter region of the *DEFB1* and *CAMP* genes that have been predicted to modify the recognition site of a transcription factor that was supposed to interact in this portion of the DNA and thus the transcription rate is altered [3]. This could lead to a deficient host immune response that could induce susceptibility to infection and disease. Some SNPs in the promoter of the *DEFB1* gene have been associated to several inflammatory, allergic, and infectious diseases including TB [3,31].

hBD-1 elicitors have been suggested as an immunotherapeutic and as excellent candidates to the development of vaccines against pulmonary TB, Crohn's disease, asthma, chronic obstructive pulmonary disease and others, because it enhances the T_H1 cells response during bacterial invasion and the production of cytokines as TNF- α , IL-12 and IFN- γ , essential elements in the efficient immune response against Mtb invasion [56]. For such reasons, it has been considered that hBD-1 could be the most relevant AP in the epithelial defense against infections.

CONCLUSION

There are many other genes that have been found to be related to TB [55,57-60] that are not mentioned above. However we have made emphasis in genes involved in the immune response because the genetic associations could help us understand the molecular basis of the TB pathogenesis. By understanding the genes involved by these genetic association studies, it could help researchers to create novel hypotheses and projects that could lead to a deeper molecular comprehension of TB by using a systems biology perspective of the host-pathogen interaction dynamics. This knowledge enhances new fields of clinical applications such as personalized medicine.

References

1. Jordao L, Vieira OV. Tuberculosis: new aspects of an old disease. *Int J Cell Biol*. 2011; 2011: 403623.
2. World Health Organization. Global Tuberculosis report 2014. WHO Library Cataloguing, Geneva. 2014.

3. Flores Saiffe Fariás A, Jaime Herrera López E, Moreno Vázquez CJ, Li W, Prado Montes de Oca E Predicting functional regulatory SNPs in the human antimicrobial peptide genes DEFB1 and CAMP in tuberculosis and HIV/AIDS. *Comput Biol Chem.* 2015; 59: 117-125.
4. Cooper AM. Cell-mediated immune responses in tuberculosis. *Annu Rev Immunol.* 2009; 27: 393-422.
5. O'Garra A. Systems approach to understand the immune response in tuberculosis: an iterative process between mouse models and human disease. *Cold Spring Harb Symp Quant Biol.* 2013; 78: 173-177.
6. Alcaïs A, Quintana-Murci L, Thaler DS, Schurr E, Abel L, Casanova JL. Life-threatening infectious diseases of childhood: single-gene inborn errors of immunity? *Ann N Y Acad Sci.* 2010; 1214: 18-33.
7. Thoma-Uszynski S, Stenger S, Takeuchi O, Ochoa MT, Engele M, Sieling PA, et al. Induction of direct antimicrobial activity through mammalian toll-like receptors. *Science.* 2001; 291: 1544-1547.
8. North RJ, Jung YJ. Immunity to tuberculosis. *Annu Rev Immunol.* 2004; 22: 599-623.
9. Flynn JL, Chan J. Immune evasion by *Mycobacterium tuberculosis*: living with the enemy. *Curr Opin Immunol.* 2003; 15: 450-455.
10. Podinovskaia M, Lee W, Caldwell S, Russell DG. Infection of macrophages with *Mycobacterium tuberculosis* induces global modifications to phagosomal function. *Cell Microbiol.* 2013; 15: 843-859.
11. Daniel J, Maamar H, Deb C, Sirakova TD, Kolattukudy PE. *Mycobacterium tuberculosis* uses host triacylglycerol to accumulate lipid droplets and acquires a dormancy-like phenotype in lipid-loaded macrophages. *PLoS Pathog.* 2011; 7: e1002093.
12. Verhagen LM, Zomer A, Maes M, Villalba JA, Del Nogal B, Eleveld M, et al. A predictive signature gene set for discriminating active from latent tuberculosis in Warao Amerindian children. *BMC Genomics.* 2013; 14: 74.
13. Xu S, Cooper A, Sturgill-Koszycki S, van Heyningen T, Chatterjee D, Orme I, et al. Intracellular trafficking in *Mycobacterium tuberculosis* and *Mycobacterium avium*-infected macrophages. *J Immunol.* 1994; 153: 2568-2578.
14. Yang CS, Yuk JM, Jo EK. The role of nitric oxide in mycobacterial infections. *Immune Netw.* 2009; 9: 46-52.
15. Nathan C. Inducible nitric oxide synthase in the tuberculous human lung. *Am J Respir Crit Care Med.* 2002; 166: 130-131.
16. Jordao L, Bleck CK, Mayorga L, Griffiths G, Anes E. On the killing of mycobacteria by macrophages. *Cell Microbiol.* 2008; 10: 529-548.
17. Lee JS, Yang CS, Shin DM, Yuk JM, Son JW, Jo EK. Nitric Oxide Synthesis is Modulated by 1,25-Dihydroxyvitamin D3 and Interferon-gamma in Human Macrophages after Mycobacterial Infection. *Immune Netw.* 2009; 9: 192-202.
18. Hmama Z, Gabathuler R, Jefferies WA, de Jong G, Reiner NE. Attenuation of HLA-DR expression by mononuclear phagocytes infected with *Mycobacterium tuberculosis* is related to intracellular sequestration of immature class II heterodimers. *J Immunol.* 1998; 161: 4882-4893.
19. Ting LM, Kim AC, Cattamanchi A, Ernst JD. *Mycobacterium tuberculosis* inhibits IFN-gamma transcriptional responses without inhibiting activation of STAT1. *J Immunol.* 1999; 163: 3898-3906.
20. Wojciechowski W, DeSanctis J, Skamene E, Radzioch D Attenuation of MHC class II expression in macrophages infected with *Mycobacterium bovis* bacillus Calmette-Guérin involves class II transactivator and depends on the Nramp1 gene. *J Immunol.* 1999; 163: 2688-2696.
21. Yoshida A, Inagawa H, Kohchi C, Nishizawa T, Soma G. The role of toll-like receptor 2 in survival strategies of *Mycobacterium tuberculosis* in macrophage phagosomes. *Anticancer Res.* 2009; 29: 907-910.
22. Yates RM, Hermetter A, Taylor GA, Russell DG. Macrophage activation downregulates the degradative capacity of the phagosome. *Traffic.* 2007; 8: 241-250.
23. Padhi A, Sengupta M, Sengupta S, Roehm KH, Sonawane A. Antimicrobial peptides and proteins in mycobacterial therapy: current status and future prospects. *Tuberculosis (Edinb).* 2014; 94: 363-373.
24. Liu PT, Stenger S, Tang DH, Modlin RL. Cutting edge: vitamin D-mediated human antimicrobial activity against *Mycobacterium tuberculosis* is dependent on the induction of cathelicidin. *J Immunol.* 2007; 179: 2060-2063.
25. Hmama Z, Sendide K, Talal A, Garcia R, Dobos K, Reiner NE. Quantitative analysis of phagolysosome fusion in intact cells: inhibition by mycobacterial lipoarabinomannan and rescue by an 1 α ,25-dihydroxyvitamin D3-phosphoinositide 3-kinase pathway. *J Cell Sci.* 2004; 117: 2131-2140.
26. Sun CQ, Arnold R, Fernandez-Golarz C, Parrish AB, Almekinder T, He J, et al. Human beta-defensin-1, a potential chromosome 8p tumor suppressor: control of transcription and induction of apoptosis in renal cell carcinoma. *Cancer Res.* 2006; 66: 8542-8549.
27. Han Q, Wang R, Sun C, Jin X, Liu D, Zhao X, et al. Human beta-defensin-1 suppresses tumor migration and invasion and is an independent predictor for survival of oral squamous cell carcinoma patients. *PLoS One.* 2014; 9: e91867.

28. Zhu BD, Feng Y, Huang N, Wu Q, Wang BY. Mycobacterium bovis bacille Calmette-Guérin (BCG) enhances human beta-defensin-1 gene transcription in human pulmonary gland epithelial cells. *Acta Pharmacol Sin.* 2003; 24: 907-912.
29. Lee SW, Chuang TY, Huang HH, Lee KF, Chen TTW, Kao YH, et al. Interferon gamma polymorphisms associated with susceptibility to tuberculosis in a Han Taiwanese population. *J Microbiol Immunol Infect.* 2014; 1-5.
30. Zhang G, Chen X, Chan L, Zhang M, Zhu B, Wang L, et al. An SNP selection strategy identified IL-22 associating with susceptibility to tuberculosis in Chinese. *Sci Rep.* 2011; 1: 20.
31. López Campos GN, Velarde Félix JS, Sandoval Ramírez L, Cázares Salazar S, Corona Nakamura a L, Amaya Tapia G, et al. Polymorphism in cathelicidin gene (CAMP) that alters Hypoxia-inducible factor (HIF-1α:ARNT) binding is not associated with tuberculosis. *Int J Immunogenet.* 2014; 41: 54-62.
32. Png E, Alisjahbana B, Sahiratmadja E, Marzuki S, Nelwan R, Balabanova Y, et al. A genome wide association study of pulmonary tuberculosis susceptibility in Indonesians. *BMC Med Genet.* 2012; 13: 5.
33. Thye T, Owusu-Dabo E, Vannberg FO, van Crevel R, Curtis J, Sahiratmadja E, et al. Common variants at 11p13 are associated with susceptibility to tuberculosis. *Nat Genet.* 2012; 44: 257-259.
34. Mahasirimongkol S, Yanai H, Mushiroda T, Promphittayarat W, Wattanapokayakit S, Phromjai J, et al. Genome-wide association studies of tuberculosis in Asians identify distinct at-risk locus for young tuberculosis. *J Hum Genet.* 2012; 57: 363-367.
35. Lu C, Wu J, Wang H, Wang S, Diao N, Wang F, et al. Novel biomarkers distinguishing active tuberculosis from latent infection identified by gene expression profile of peripheral blood mononuclear cells. *PLoS One.* 2011; 6: e24290.
36. Rossouw M, Nel HJ, Cooke GS, van Helden PD, Hoal EG. Association between tuberculosis and a polymorphic NFκB binding site in the interferon gamma gene. *Lancet.* 2003; 361: 1871-1872.
37. Pacheco AG, Cardoso CC, Moraes MO. IFNG +874T/A, IL10 -1082G/A and TNF -308G/A polymorphisms in association with tuberculosis susceptibility: a meta-analysis study. *Hum Genet.* 2008; 123: 477-484.
38. Jouanguy E, Dupuis S, Pallier A, Dörfinger R, Fondanèche MC, Fieschi C, et al. In a novel form of IFN-gamma receptor 1 deficiency, cell surface receptors fail to bind IFN-gamma. *J Clin Invest.* 2000; 105: 1429-1436.
39. Storgaard M, Varming K, Herlin T, Obel N. Novel mutation in the interferon-gamma-receptor gene and susceptibility to mycobacterial infections. *Scand J Immunol.* 2006; 64: 137-139.
40. Cooke GS, Campbell SJ, Sillah J, Gustafson P, Bah B, Sirugo G, et al. Polymorphism within the interferon-gamma/receptor complex is associated with pulmonary tuberculosis. *Am J Respir Crit Care Med.* 2006; 174: 339-343.
41. Flynn JL, Goldstein MM, Chan J, Triebold KJ, Pfeffer K, Lowenstein CJ, et al. Tumor necrosis factor-α is required in the protective immune response against mycobacterium tuberculosis in mice. *Immunity.* 1995; 2: 561-572.
42. Stein CM, Nshuti L, Chiunda AB, Boom WH, Elston RC, Mugerwa RD, et al. Evidence for a major gene influence on tumor necrosis factor-α expression in tuberculosis: path and segregation analysis. *Hum Hered.* 2005; 60: 109-118.
43. Correa PA, Gomez LM, Cadena J, Anaya JM. Autoimmunity and tuberculosis. Opposite association with TNF polymorphism. *J Rheumatol.* 2005; 32: 219-224.
44. Amirzargar AA, Rezaei N, Jabbari H, Danesh AA, Khosravi F, Hajabdolbaghi M, et al. Cytokine single nucleotide polymorphisms in Iranian patients with pulmonary tuberculosis. *Eur Cytokine Netw.* 2006; 17: 84-89.
45. Awomoyi AA, Charurat M, Marchant A, Miller EN, Blackwell JM, McAdam KP, et al. Polymorphism in IL1B: IL1B-511 association with tuberculosis and decreased lipopolysaccharide-induced IL-1β in IFN-gamma primed ex-vivo whole blood assay. *J Endotoxin Res.* 2005; 11: 281-286.
46. Larcombe LA, Orr PH, Lodge AM, Brown JS, Dembinski IJ, Milligan LC, et al. Functional gene polymorphisms in canadian aboriginal populations with high rates of tuberculosis. *J Infect Dis.* 2008; 198: 1175-1179.
47. Ma X, Reich RA, Wright JA, Tooker HR, Teeter LD, Musser JM, et al. Association between interleukin-8 gene alleles and human susceptibility to tuberculosis disease. *J Infect Dis.* 2003; 188: 349-355.
48. Delgado JC, Baena A, Thim S, Goldfeld AE. Ethnic-specific genetic associations with pulmonary tuberculosis. *J Infect Dis.* 2002; 186: 1463-1468.
49. Ma X, Liu Y, Gowen BB, Graviss EA, Clark AG, Musser JM. Full-exon resequencing reveals toll-like receptor variants contribute to human susceptibility to tuberculosis disease. *PLoS One.* 2007; 2: e1318.
50. Ogus AC, Yoldas B, Ozdemir T, Uguz A, Olcen S, Keser I, et al. The Arg753Gln polymorphism of the human toll-like receptor 2 gene in tuberculosis disease. *Eur Respir J.* 2004; 23: 219-223.
51. Newport MJ, Allen A, Awomoyi AA, Dunstan SJ, McKinney E, Marchant A, et al. The toll-like receptor 4 Asp299Gly variant: no influence on LPS responsiveness or susceptibility to pulmonary tuberculosis in The Gambia. *Tuberculosis (Edinb).* 2004; 84: 347-352.

52. Davila S, Hibberd ML, Hari Dass R, Wong HE, Sahiratmadja E, Bonnard C, et al. Genetic association and expression studies indicate a role of toll-like receptor 8 in pulmonary tuberculosis. *PLoS Genet.* 2008; 4: e1000218.
53. Velez DR, Wejse C, Stryjewski ME, Abbate E, Hulme WF, Myers JL, et al. Variants in toll-like receptors 2 and 9 influence susceptibility to pulmonary tuberculosis in Caucasians, African-Americans, and West Africans. *Hum Genet.* 2010; 127: 65-73.
54. Gombart AF, Borregaard N, Koeffler HP. Human cathelicidin antimicrobial peptide (CAMP) gene is a direct target of the vitamin D receptor and is strongly up-regulated in myeloid cells by 1,25-dihydroxyvitamin D3. *FASEB J.* 2005; 19: 1067-1077.
55. Azad AK, Sadee W, Schlesinger LS. Innate immune gene polymorphisms in tuberculosis. *Infect Immun.* 2012; 80: 3343-3359.
56. Prado Montes de Oca E. Antimicrobial peptide elicitors: new hope for the post-antibiotic era. *Innate Immun.* 2013; 19: 227-241.
57. Yim JJ, Selvaraj P. Genetic susceptibility in tuberculosis. *Respirology.* 2010; 15: 241-256.
58. Fernando SL, Britton WJ. Genetic susceptibility to mycobacterial disease in humans. *Immunol Cell Biol.* 2006; 84: 125-137.
59. Thuong NT, Dunstan SJ, Chau TT, Thorsson V, Simmons CP, Quyen NT, et al. Identification of tuberculosis susceptibility genes with human macrophage gene expression profiles. *PLoS Pathog.* 2008; 4: e1000229.
60. McKusick VA. Mendelian Inheritance in Man and its online version, OMIM. *Am J Hum Genet.* 2007; 80: 588-604.