Mycobacterium Tuberculosis Infection: Participation of TH1, TH2, TH17 and Regulatory T Cells in the Immune Response
Gerardo Fernando Fernández Soto1,3,4, Nereida Valero Cedeño1, Carolina Arráiz de Fernández1, Patricia Paredes Lascano2,4, Miriam Fernández Nieto1, María Teresa Peñaherrera Ron1
1 Nursery Career, School of Health Sciences, Ambato Technical University, Ambato, Ecuador
2 Medicine Career, School of Health Sciences, Ambato Technical University, Ambato, Ecuador
3 Early Stimulation Career, School of Health Sciences, Ambato Technical University, Ambato, Ecuador
4 Pediatric Service of the Ambato General Hospital, Ambato, Ecuador

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Abstract- Mycobacterium tuberculosis, the etiologic agent of Tuberculosis, is a pathogen that is widely distributed geographically. Tuberculosis is classified as a granulomatous inflammatory condition where effector cells accumulate at the site of mycobacterial infection to form the characteristic tubercle. Regulating proteins of Th1 and Th17 cells participate in the formation of Mycobacterium-induced granuloma. The predominance of Th2 phenotype cytokines increases the severity of Tuberculosis. Treg cells are increased in patients with active Tuberculosis but decrease with anti-Tuberculosis treatment. The increment of these cells causes down-regulation of adaptive immune response facilitating the persistence of the bacterial infection. Mycobacterium tuberculosis-induced Treg cells to secrete cytokines that inhibit the immune response. This has been considered an important evasion mechanism although it is not the only that intervenes. The evolution of the Mycobacterium tuberculosis infection will depend on the cytokines' network that traduces pathological change in cells and tissues which explain the clinical manifestations existing in affected patients.

Keywords: Mycobacterium tuberculosis; Virulence; Host genetic; Immune response; Cytokines

Introduction

Tuberculosis (TB) is one of the leading causes of mortality and morbidity in different age groups throughout the world, especially in developing countries. TB is classified as a granulomatous inflammatory condition where effector cells accumulate at the site of mycobacterial infection to form the characteristic tubercle. Bacterial virulence factors, nutritional state, host genetic condition, and immune response play an important role in the evolution of the infection (1). Mycobacterium tuberculosis (Mt) enters the body by the airway (95%). It is usually located in the lungs, TB, but can spread through the blood and it produces extrapulmonary tuberculosis, with the involvement of the lymph nodes, pleura, genitourinary system, meninges, and peritoneum. Ninety percent of infected individuals will remain latently infected without clinical symptom, however, 10% of the individuals infected with Mt will develop the active disease (2).

Mycobacteria persistence is associated with failure in the immune vigilance and reactivation of the disease among other factors. Many cells such as neutrophils, macrophages, dendritic cells, B-Lymphocytes, and NK cells take place in the immune response in the Mt infection. The cytokines that are secreted from these cells form a complex network that traduces pathological change in cells and tissues.

The genetically diverse Mt strains from different lineages have been shown to induce variable immune system response. The establishment of the infection determines, with the time of evolution, the development of the active and latent forms (1). We discuss the factors related to immune response and the participation of cells and regulating proteins in the Mt infection. Furthermore, overall information inherent to the behavior of cytokines
that allow explaining the clinical manifestations and the evolution of the disease among other aspects, that represents a substantial contribution to the knowledge of TB will be discussed.

**Immune response: Participation of Th1, Th2, Th17, and regulatory T cells**

Innate and adaptive immune responses are important for the eradication of the microorganism. Armed effector T cells are crucial to almost all adaptive immune response. Alterations of the T helper cells functions conduce to inefficient clearance of pathogens and can cause inflammation and autoimmunity (3). The reasons for the impaired Mtb-specific T cell function in active tuberculosis remain controversial. When mutations in Th-1 cytokine signaling pathways (Interferon-γ and Interleukin-12) are present, the patients are susceptible to overwhelming infection with Mtb (4,5). IFN-γ is a critical mediator of macrophage activation and bactericidal mechanisms. Impaired Th1 lymphocyte response in HIV infection also produces ineffective immunity to Mtb. Latent TB infection, reactivation and advanced TB are associated with predominance of Th2 cytokines (Interleukin-4 and interleukin-10) (6-8).

Patients with extrapulmonary disease have immune responses in vitro suggestive of Th1 response, whereas patients with the military/disseminated disease have a suggestive Th2 response (9). Observations appoint that virulent Mtb strains preferentially induce Th2 cytokines expression, whereas less virulent strains induce Th1 cytokines, including interferon-γ and tumor necrosis factor-α (10-12). Overexpression of Th2 cytokines increases the severity of TB.

The etiological agent and its immunogenicity, evasion mechanisms of the pathogen, concurrent infections and infestations, the host genetic condition and the sufficiency or insufficiency of the immune system, are factors that can change the immune response in different pathological entities such as Helicobacter pylori and Mycobacterium tuberculosis infection among others (1,13). Coincident hookworm infection exerts a profound inhibitory effect on protective Th1 and Th17 response in latent TB and may predispose toward the development of active TB in humans (14).

In the antigenic presentation, the function of MHC class II molecules is to present peptides generated in the intracellular vesicles of B cells, macrophages, and other antigen-presenting cells to CD4 T cells. CD4+T cells are required for the control of intracellular Mtb. There is no doubt that immunity to Mtb depends on Th1-cell activity (Interferon-γ and Interleukin-12 and the production of tumor necrosis factor- α, but Th1 immunity alone is not sufficient to protect the host from Mtb infection, development of the disease, or dissemination (15). For other authors, active TB is characterized by a profound and prolonged suppression of Mtb-specific T cell responses, as evidenced by the decreased production of the Th1 phenotype cytokines as interleukin-2 and interferon-γ (16-20).

Incremented secretion of immunosuppressive cytokines (interleukin-10 and transforming growth factor-β) by mononuclear phagocytes has been implicated in decreased T cell function during TB (21-24). Other studies are controversial with respect to Interferon-γ serum levels. These studies reported significantly higher active TB levels in patients after treatment than in patients during anti- TB therapy, patients with history of TB contact and in healthy control. Also, they observed the increment of interleukin-10, Interleukin-6, and decrease of Interleukin-4 (24,25).

The predominance of Th1 phenotype plays a relevant role in immunity to TB in children. The children are more prone to developing extrapulmonary manifestations of TB than adults. Pediatric TB is characterized by diminished Th1, Th2, and 17 phenotype cytokines, which favor the development of neurologic TB, suggesting a crucial role for these cytokines in protection against pediatric tuberculosis. Among children with extrapulmonary TB, those with neurologic involvement exhibited a more significantly diminished Ag-driven Interferon-γ and Interleukin-17 production (26).

Regulatory T cells (Treg) are implicated in the pathogenesis of Mtb infection. The induced Treg cells (iTreg) are differentiated from naïve T cells in the presence of Transforming growth factor β following T cell receptor (TCR) stimulation. These cells produce large amounts of Interleukin-10 and Transforming growth factor-β (27,28). Unlike Th1, Th2 or Th17 cells, iTreg displays immune-suppressive activity with minimal antigen specificity (28). Tregs are increased in the peripheral blood of active TB patients compared with M. bovis BCG vaccinated healthy donors. This agrees with recent reports in humans (30) and in the murine TB model (31,32). It has been demonstrated that Treg cells proliferate and accumulate at sites of infection and have the capacity to suppress immune responses (32). Circulating Treg cells in the peripheral blood declined progressively by anti-TB treatment (33). During the initial T cell response to Mtb infection, the pathogen induces the expansions of Treg cells that delay the onset of adaptive immunity, suggesting that Mtb has sequestered Treg to allow the bacterium replicate
endlessly in the lungs until T cells finally arrive. The induction of Treg by Mtb can be an evasive mechanism of the bacterium that permits its replication (34). The increase of these cells causes down-regulation of adaptive immune response facilitating the persistence of bacterial infections (34,35). Studies have appreciated high levels of circulating Treg cells in active TB infection which inhibit the TH1 response but not the TH17, facilitating the bacterial replication and tissue damage.

The presence of persisting immune activation and high frequencies of Treg lymphocytes may reflect immune dysregulation that predisposes individuals to clinical tuberculosis, specifically to extrapulmonary TB (36,37). There are high levels of transforming growth factor-β that produce active immunosuppression at the local infection site. These results suggest that an imbalance in the proportion of effector T cells to Treg cells, at the site of infection, may contribute to the establishment of TB infection (38). CD4, CD8, and γδ T- and B-lymphocytes among other cells produce their own complement of chemokines and cytokines, which amplify cellular recruitment and remodeling of the infection site into the granuloma.

Persistent TNF-α production is required to sustain the chemokine gradient and maintain the structure of the granuloma (39). Mtb has many strategies to evade the immune response and to survive within the macrophage in a bacterial-immunological equilibrium. Mtb manipulates different elements of immunity system during “establishment, maintenance and necrotic liquefaction of the granuloma facilitating transmission” and alters the host cell cycle to promote long-term persistence (40).

There are many factors that can change the immune response in different pathologies, such as: the etiological agent and its immunogenicity, evasion mechanisms of the pathogen, the type of pathology, the phase of the clinical entity, concurrent infections and infestations, the host genetic condition and the sufficiency or insufficiency of the immune system among others (1,13). All this determine the contradictions of some studies due to the quantity of variables that influence the immune system. The infection by Mtb does not escape all of the pointed considerations (13).

**Genetic susceptibility to tuberculosis**

Substantial evidence now regards the human genetic contribution to susceptibility to tuberculosis. This evidence has come from several whole-genome linkage scans and numerous case-control association studies where the candidate genes were derived from the genome screens, animal models and hypotheses pertaining to the disease pathways. Human factors governing whether the infected individual will progress to active tuberculosis disease or not are usually assumed to be those governing the immunological state of the host, which are generally determined by the host’s genetic makeup (41,42). Several studies to date have proven that genetic factors contribute to the outcome of tuberculosis, with an estimated heritability ranging from 36% to 80% (43,44,45,46,47).

Tuberculosis disease in a family does not follow a Mendelian pattern and is polygenic and multifactorial. Genetic susceptibility studies in tuberculosis are additionally complicated by the presence of two different genomes, the bacterium, and the host, and the influence their interaction can have on the disease (48).

Numerous studies have identified mostly in genes that are vital for immunity against intracellular pathogens in the interleukin (IL)-12/IL-23/interferon Interferon-γ axis (49). Several studies regarding the role of immune system-related genes have been performed. The human leucocyte antigen (HLA) region consists of approximately 200 genes that it has been postulated to affect infectious disease (50). HLA genes have been examined in several tuberculosis susceptibility studies and were some of the first genes to be investigated and associated with the disease. The HLA-DR2 is most consistently associated with TB in several populations such as India (51,52,53,54,55). It was found that the HLA-DQB10503 influence the progression of TB in the Cambodian population (56), whereas the DQB10601 was associated with TB in the Thai and South Indian population (52,53). DRB11302 was associated with TB susceptibility in a Venda population (50).

NRAMP1 is a divalent transporter localized to the late endosomal membrane that regulates cytoplasmic cation levels by specifically regulating the iron metabolism in the macrophages, leading to the possible containment of early mycobacterial infections (57). Genetic variants of NRAMP1 have been associated with TB in several studies (58,59,60), leading to the meta-analysis of NRAMP1 polymorphisms, which confirmed their involvement in TB susceptibility (60).

The Interferon-γ pathway is one of the most well known in TB because it is a flagship Th1 cytokine and plays a vital role in the protective immune response against Mtb infection (61). Several polymorphisms have been identified in Interferon-γ and in the alpha and beta chains of the Interferon-γ receptor (IFNGR) gene that was mapped to chromosome 12 and 6, respectively (62,63). The functional Interferon-γ and IFNGR form a vital complex essential for containing Mtb (62) One of the
most studied polymorphisms in Interferon-γ is located in the first intron (1874 T/A) and has been associated with TB susceptibility in several populations such as Spanish (64,65,66).

The nitric oxide synthase 2A gene (NOS2A) is induced in response to infections and cytokines and produces the inducible nitric oxide synthase protein (67). Among its other biological functions, nitric oxide (NO) has been recognized as a mediator of immunity to TB (68). The specific mechanism by which NO controls Mtb is not clear, but may involve disruption of bacterial DNA, proteins, signaling and/or induction of apoptosis of macrophages that contain the bacterium (69). It may also play a role in the formation of protective granulomas (70).

Two SNPs (rs2779249 and rs2301369) in the promoter of NOS2A were associated with TB in a family-based study in 92 Brazilian families. NOS2A microsatellite promoter polymorphism was associated with TB in Colombia (71) and in the South African colored population.

Chemokines play an important role in the development of immune responses against tuberculosis. The C-C chemokine ligand-2 gene (CCL2) encodes monocyte chemoattractant protein-1 (MCP-1), which is essential for the recruitment of monocytes, T lymphocytes (72), and natural killer cells (73) to the site of mycobacterial infection. It may also take part in the localization of TB in the lungs by contributing to granuloma formation (72) and possibly have a role in T cell differentiation (74). The functional CCL2 promoter polymorphism rs1024611 (75) was associated with increased susceptibility to pulmonary TB in Mexicans and in Koreans (76). However, this polymorphism was not associated with TB in a smaller study of Brazilians (77) or in a large case-control association study in the South African colored population (78).

The family of mammalian Toll-like receptors (TLRs) consists of at least 13 proteins, each with a distinct function, which initiates the innate immune response. TLRs are central components of the innate immune response to mycobacterial infection (79,80)) and act as part of the pattern recognition system to signal the presence of Mtb in the host (81). Two common nonsynonymous polymorphisms in TLR1, namely rs4833095 (N248S) and rs5743618 (I602S), have been implicated in TB susceptibility in the African–American population (82). The rs5743708 SNP (Arg753Gln) was investigated in Turkey, and it was suggested that it might contribute to the risk of developing tuberculosis (83).

The gene encoding the collectin mannose-binding lectin (MBL) or mannos-binding protein (MBP) maps to chromosome 10. MBL is a serum lectin that acts as an opsonin to promote phagocytosis and modulates inflammation (84,85), and the variant alleles have been associated with protection against tuberculosis and particularly tuberculous meningitis in South Africa and Turkey (86,87,88). The transmembrane C-type lectin, dendritic cell-specific intracellular adhesion molecule (ICAM)-grabbing nonintegrin (DC-SIGN), or CD209, located on chromosome 19, is known to be the major M. tuberculosis receptor in human dendritic cells, and as such, functions in the pulmonary innate immune system (89). Two promoter variants, 871 A/G and 336 A/G, have been studied regarding susceptibility to tuberculosis in South Africa and the 871G/ 336A haplotype is significantly more frequent among healthy controls (90).

The vitamin D receptor gene (VDR) mediates the effects of the active metabolite of vitamin D, 1,25-dihydroxyvitamin D3, which suppresses the growth of Mtb in vitro (91,92) by stimulating cell-mediated immunity and activating monocytes (93). Conflicting results have been found in studies of VDR in TB (95,96). A recent study in South Africans determined that the Apal ‘AA’ genotype and ‘T’-containing TaqI genotypes predicted a faster response to TB treatment but did not detect an association with TB in a case-control analysis (97).

**T cells in active and non-active tuberculosis**

Early diagnosis of active TB followed by short-term anti-TB treatment remains a major strategy for TB control. The detection of the pathogen and Interferon-gamma (IFN-γ) release assays (IGRAs) for immune-diagnosis of its infection have been developed as the most important achievements over decades and are increasingly applied (98). IGRAs have no issues in sample preparation but cannot distinguish active TB from latent TB (LTB). This is a major defect that limits its clinical application because the treatments for active TB are considerably different from those of LTBI. Several additional markers have been explored to work in combination with IFN-γ, and some have produced considerable diagnostic improvements (99,100,101).

CD161-expressing T cells-indices are useful to discriminate active TB from LTB. Besides CD4 and CD8 (101,102), the immunological markers implicated in identification of active TB also include CD38, CD26, CD27, CD64 and CD161 (99,100,103,104). CD161 is a C-type lectin-like receptor expressed by a broad range of lymphocytes, including CD4+, CD8+, γδ+ T-cells, NK cells, and mucosal-associated invariant T (MAIT) cells. Consistent with the extraordinary heterogeneity of CD161-expressing cells, the biological functions of the
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marker including its roles in mycobacterial infections are not well understood. Nevertheless, the decrease of CD161-expressing CD4 T cells in active TB patients was in line with the suppressed Th17 responses in the disease (105,106,107), in which, Th17 cells were CD161-positive (106,107). MAIT cells (CD161 positive) represent the most abundant innate-like T-cell population within the human body, comprising up to ~5% of the total T-cell population and are involved in anti-bacterial immunity (108).

Recently, it was shown that patients with active tuberculosis had significantly lower percentage of CD161-expressing CD8+TRAV1–2+ MAIT cells than those of uninfected and LTB subjects (109,110). This evidence suggest that CD161-expressing lymphocytes are important for protective immunity in preventing the progression of active TB from Mtb infection.

Studies reveal that cytokines' network is formed with the participation of the regulating proteins and different subset of cells to achieve control, persistence, and severity of TB. The synergistic, antagonistic, redundant and pleiotropic biological effects of regulating proteins can affect or not the immune response against Mtb. There is no doubt that immunity to Mtb depends on Th1-cell activity (IFN-γ and IL-12 and the production of TNF-α), but Th1 immunity alone is not sufficient to protect the host from Mtb infection, development of the disease, or dissemination. The predominance of Th2 phenotype cytokines (Interleukin-10 and Transforming growth factor β) increases the severity of TB. Treg cells that secrete immunosuppressive cytokines are increased in patients with active TB but decrease with anti-TB treatment. Mtb is considered a manipulator of protective immunity. The balance between host immunity and bacterial evasion strategies among other factors determine the control in vivo of Mtb. The intricate complexity of cytokines and chemokines stimulate the search for more effective therapeutics that permit the eradication of a disease such as TB.

The human factors governing whether the infected individual will progress to active TB disease or not are usually assumed to be those governing the immunological state of the host, which are generally determined by the host’s genetic makeup, for example, genetic variants of NRAMP1 have been associated with TB, because that specifically regulate the iron metabolism in the macrophages, leading to possible containment of early mycobacterial, involvement in TB susceptibility, also the IFN-γ pathway is one of the most well known in TB because it is a flagship Th1 cytokine and plays a vital role in the protective immune response against Mtb infection. Several polymorphisms have been identified in IFNG and in the a and b chains of the IFN-γ receptor gene that was mapped to chromosome 12 and 6.

The detection of the pathogen, and IFN-γ release assays for immune-diagnosis of its infection, have been developed as the most important achievements over decades and are increasingly applied, IGRAs have no issues in sample preparation but cannot distinguish active TB from latent TB (LTB), the immunological markers implicated in identification of active TB include CD161, CD38, CD26, CD27, and CD64.

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References

8. Senait Ashenafi, Getachew Aderaye, Amsalu Bekele,


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58. Huang L, Liu C, Liao G, Yang X, Tang X, Chen J. Vitamin D Receptor Gene FokI Polymorphism Contributes to Increasing the Risk of Tuberculosis: An Update Meta-
Mycobacterium tuberculosis infection


