

T Cell Mediated Immune Responses Against Mycobacterium Tuberculosis

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ABSTRACT

One-third of the world's population is estimated to be infected with *Mycobacterium tuberculosis* (**M. tuberculosis**) and remains asymptomatic. Of these, only 5-10% of the individuals are known to develop active tuberculosis in their lifetime. Host T cell mediated adaptive immune responses are of critical importance against *M. tuberculosis* infection. However the immune mechanisms that determine why some individuals are protected from disease while others develop infection are yet to be defined. This chapter aims to summarize the role of different T cell subsets against and their regulation against *M. tuberculosis* infection.

INTRODUCTION

The outcome of an infection with *M. tuberculosis* depends on the effect balance between the host innate and adaptive immune response. In most cases, the interaction between *M. tuberculosis* and the alveolar macrophage results in clearance of the bacillus. Multiple factors are involved in achieving this response and this chapter focuses on the role of adaptive immunity in mycobacterial clearance.

ADAPTIVE IMMUNITY

Cell mediated immunity (**CDI**) plays a central role in protection against tuberculosis. Since *M. tuberculosis* is an intracellular pathogen and primarily resides in macrophages, therefore T cell effector mechanism rather than antibodies are required to clear the infection [1]. The interaction between infected macrophages and T lymphocytes determines the outcome of infection. Among T lymphocytes, CD4 and CD8 cells are essential for the host defense against tuberculosis. Naive CD4 and CD8 T cells encounter the bacilli when infected macrophages or antigen presenting cells interact with T cells in secondary lymphoid organs and become activated. Activated T cells then migrate via blood stream to the site of infection where they secrete cytokines or kill the infected cells via direct contact [2].

CD4 T CELLS

CD4 T cells express T cell receptors (**TCR**) that recognizes antigens in context of MHC class II molecules present on the surface of antigen presenting cells such as monocytes, macrophages, or dendritic cells [3]. In murine models, one to three weeks after infection, *M. tuberculosis* specific CD4 T cells appear. Studies have shown that CD4 depleted mice are unable to clear *M. tuberculosis* infection and succumb to it [4]. CD4 in response to exposure with *M. tuberculosis* antigens secrete the pro-inflammatory cytokine IFN- γ , which is known to be associated with increased protection by activating reactive oxygen and nitrogen intermediates, bringing about the killing of *M. tuberculosis* [5]. An increase in the number of activated CD4 T effectors cells may improve clearance of infection [6], while impaired activation of CD4 T cells may contribute to the persistence of TB infection. CD4 helper T-cells can be separated into at least distinct phenotypic classes, Th1 and Th2. Their differentiation from these precursor cells may be under the control of cytokines such as Interleukin-12 (**IL-12**) [7]. Phenotypically, Th1 cells are characterized mainly by their ability to produce the cytokines IFN- γ and IL-2 [8], whereas Th2 cells produce cytokines such as IL-4, IL-5, and IL-10 [9]. To maintain normal immune response, the coordinated function of both Th1 and Th2 arms of the immune system are required. Th1 cells play a role in destruction of intracellular pathogens such as *M. tuberculosis*, *M. leprae* and *C. pneumoniae*. However, if left unregulated exacerbated Th1 responses may lead to Th1 dominated disorders such as autoimmune disorders as well as chronic inflammatory disorders [10]. Th2 responses on the other hand are effective against allergic responses or helminth infections but uncontrolled rise in Th2 cells offer reduced protection against some intra-cellular pathogens, transplant tolerance and some systemic immune disorders [10].

CD8 T CELLS

CD8 T cells recognize antigens that have been processed in the cytosol and are presented in the context of MHC Class I molecules. CD8 T cells are shown to cause lysis or induce apoptosis of the infected cell [11]. CD8+ T-cells have been shown to be able to lyse *Mycobacterium*-infected macrophages resulting in the killing of the bacteria [12]. CD8 T cell depletion is also shown to

result in impaired containment of pathogen [13]. A decrease in the ratio of CD4/CD8 cells in bronchoalveolar lavage (**BAL**) cells from PTB (pulmonary TB) patients was shown, however, the ratio in peripheral blood cells was shown to be normal [14]. This reduced CD4/CD8 ratio was the consequence of CD4+ cell depletion rather than the proliferation of CD8+ cells [14]. Increased CD8+ T-cells in the BAL of patients with active tuberculosis was also reported [15]. The increase in CD8+T cells in patients with active TB is shown to be associated with increased immune protection as they play a prominent role in recognition and then ultimately lysis of cells infected with *M. tuberculosis*.

TH17 CELLS

Th17 cells are controlled by the transcription factor ROR γ t [16]. Th17 cells are mainly known to secrete IL-17 cytokine while others cytokines produced by Th17 cells are IL-22, IL-26, and GM-CSF [17]. Th17 cells are known to induce proinflammatory genes, antimicrobial peptides, recruitment and activation of neutrophils. Mainly Th17 cells protect against extracellular pathogens and mediate inflammatory response. In tuberculosis, the early recruitment of neutrophils to the site of infection is associated with early granuloma formation and efficient mycobacterial clearance [18]. In IL-17 absence, reduced numbers of neutrophils are found after BCG infection [19].

$\gamma\delta$ T CELLS

$\gamma\delta$ T cells are a subset of CD3+ T cells which contribute to a total of 2-10% of T cell population. Previous studies have shown that $\gamma\delta$ T cells are increased in patients with tuberculosis [20]. $\gamma\delta$ T cells are shown to play a critical role in immune response against *M. tuberculosis* by inhibiting its proliferation through IFN- γ secretion. $\gamma\delta$ T cells also interact with other immune cells such as NK cells, dendritic cells and CD8+ $\alpha\beta$ T cells to initiate anti-tuberculosis immune response [20]. However studies defining the role of $\gamma\delta$ T cells in immunity against *M. tuberculosis* infections are scanty.

B CELL RESPONSES

Protection against *M. tuberculosis* is often referred to as T cell-mediated, with B cells and antibodies playing a much more limited role [21]. However, several mechanisms have been proposed by which antibodies could mediate protective effects against mycobacterial infections such as to neutralize pathogen toxins, promote opsonization, and modulate complement-mediated lysis [22]. It has been shown that while mycobacteria enter the macrophages through mannose receptors, or CR1/CR3, serum antibodies IgG1 and IgG3 also play a role in opsonization and uptake of bacilli through high affinity Fc receptors [23]. Mononuclear phagocytes, in which, mycobacteria reside and multiply, have high affinity receptors (Fc γ 1 and γ 3) for IgG1 and IgG3 antibody subclasses among which IgG1 constitute the highest proportion (70%) of serum antibodies [24]. IgG1-mediated uptake of Mycobacterial antigen has been associated with the increased TNF- α secretion in advanced tuberculosis patients, linking humoral immunity and disease pathogenesis in TB [25]. This data is supported by the increased IgG1 levels found in

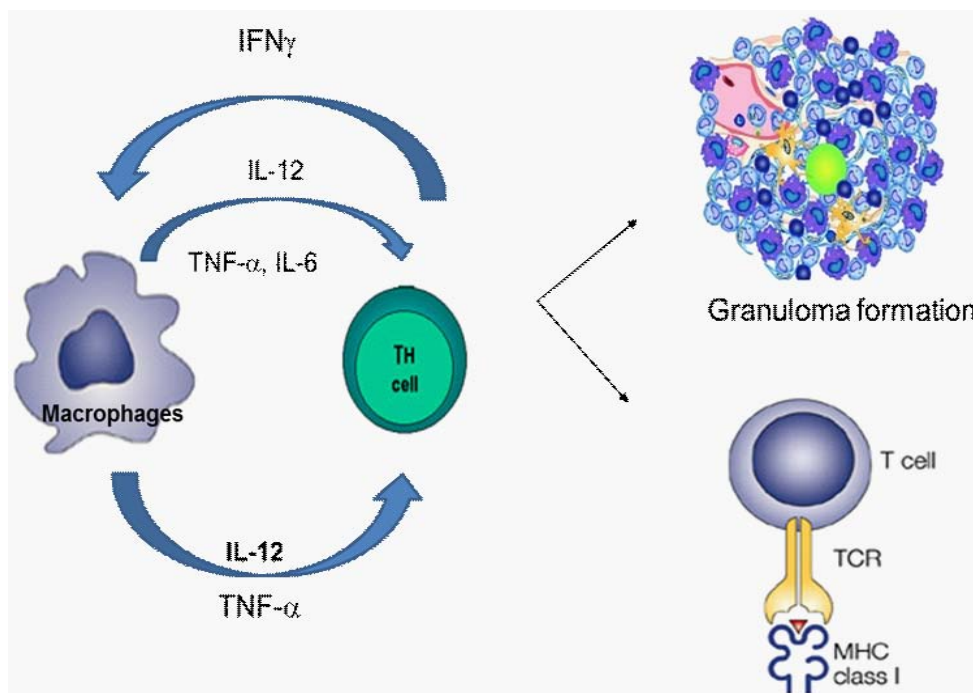
patients with TB as compared with healthy endemic controls [26]. B cells are also reported to polarize T cell responses through production of cytokines such as IL-10 which directs Th2 type responses [27]. B cells are also a major component of granuloma formation and their depletion is shown to be associated with increased pathology [22].

THE ROLE OF CYTOKINES IN TUBERCULOSIS

Cellular activation in response to *M. tuberculosis* is mediated by inflammation which is regulated through a balance of pro-inflammatory and anti-inflammatory cytokines which modulate cellular function. These may be categorized as Type 1 or Type 2 responses based on their role.

PRO-INFLAMMATORY CYTOKINES

Pro-inflammatory cytokines are defined as chemical mediators that are known to produce systemic inflammation capable of inhibiting the survival of pathogenic bacteria. Pro-inflammatory cytokines which have been shown to play an important role in outcome of tuberculosis include; IFN- γ , IL-2, IL-6 and IL-12 (Figure 1).



Antigen Presentation

Figure 1: Macrophage and T cell activation. Macrophages are activated by *M. tuberculosis* to produce cytokines such as IL-12, TNF- α and IL-6 and activate T cells that produce IFN- γ . Activated macrophages induce lymphocyte recruitment orchestrated by chemokines, leading to the formation of granulomas that contain bacilli.

IFN- γ : Th1 cells activated in response to infection with *M. tuberculosis* are known to secrete IFN- γ . IFN- γ is central for protection against tuberculosis. IFN- γ activates transcriptional expression of IFN- γ response genes mediated by the signal transducer and activator of transcription (STAT)-1 molecule [28]. IFN- γ binds to the IFN- γ cell surface receptor resulting in activation of Janus activated kinases (JAK) 1 and JAK2 which ultimately results in the phosphorylation of cytoplasmic STAT1. Tyrosine phosphorylated STAT1 homodimerizes and translocates to the nucleus. In nucleus STAT1 homodimer activates transcription of specific genes responsible for antigen presentation. IFN- γ produced by CD4 T cells activates infected macrophages, increases antigen presentation via MHC Class II molecules, and increases production of nitric oxide and reactive-oxygen intermediates [29]. Individuals with defective IFN- γ genes are more susceptible to infection with *M. tuberculosis* [30]. With regard to cytokine profiles in patients with TB, IFN- γ concentrations in peripheral blood mononuclear cells of patients are raised as compared with those in healthy persons [31]. However, in patients with advanced TB disease IFN- γ responses are shown to be down-regulated [32].

IL-2: Proliferation of T lymphocytes relies on IL-2 secretion. IL-2 is also a pre-requisite for clonal expansion of antigen specific T cells [33] and is required for the activation of cell mediated immune responses in infection with *M. tuberculosis* [34]. Previous studies have shown that defective IL-2 production may lead to defective cellular immune response in tuberculosis [34]. A 10-fold decrease in IL-2 responsive cells have been shown in tuberculosis patients as compared with healthy controls [35].

IL-6: IL-6 has properties of both pro- and anti-inflammatory cytokines. IL-6 is known to act in concert with TNF- α and IL-1 to initiate early pro-inflammatory responses [36,37], helps in the promotion of T cell and B cell responses and maintains hematopoiesis [38-40]. Studies in murine models have shown that in infection with *M. tuberculosis*, IL-6 deficiency at early stages results in increased susceptibility to infection [41,42]. However in chronic infections, IL-6 is known to have an anti-inflammatory effect. IL-6 gene up-regulation is shown to be associated with TB progression [43]. IL-6 induced by *M. tuberculosis* is known to counteract IFN- γ responses [44]. IL-6 is shown to inhibit TNF- α and IL-1 β and promote the growth of *M. avium*.

IL-12: IL-12 is secreted from macrophages and is required for the differentiation of T helper cells. IL-12 favors the development of Th1 cells and is necessary for the induction of IFN- γ responses. Increased secretion of IL-12 has been detected in lung infiltrates of PTB patients [45]. Among ETB patients, IL-12 is shown to be increased in pleural fluid [46] and also in tuberculous lymphadenitis [47]. IL-12 knockout mice show increased susceptibility to tuberculosis [48]. Individuals with mutations in genes encoding IL-12p40 and IL-12R are also shown to suffer with recurrent mycobacterial infections [49,50]. It is proposed that IL-12 plays a crucial role in mycobacterial infections by connecting innate and adaptive arm of host immune responses to clear evading pathogen [51].

ANTI-INFLAMMATORY RESPONSES

The pro-inflammatory cytokines produced by Th1 cells and macrophages are antagonized by anti-inflammatory cytokines. In tuberculosis, IL-4, IL-10 and TGF- β are shown to block the effects of protective pro-inflammatory responses.

IL-4: IL-4, is a marker of Th2 cells and shown to suppress IFN- γ production and macrophage activation. In the murine model of *M. tuberculosis* infection, IL-4 is shown to be associated with progressive disease as well as latent infection [52]. Conversely, in IL-4 knockout mice the levels of pro-inflammatory cytokines were found to be increased [53]. In human tuberculosis, increased levels of IL-4 are shown to be associated with more severe cavitory disease [54].

IL-10: IL-10 is produced by infected macrophages as well as activated T lymphocytes in tuberculosis infection [55, 56]. IL-10 is known to maintain homeostasis by counteracting the protective pro-inflammatory cytokine response by down-regulating IFN- γ , TNF- α and IL-12 [57,58]. However, in infectious diseases like tuberculosis, the IL-10 mediated suppression of pro-inflammatory cytokines may interfere with host immune responses [59]. It was shown that IL-10 transgenic mice presented with a larger bacillary burden after infection with aerosolized *M. tuberculosis* [60], while mice deficient in IL-10 presented with a low bacillary burden after infection [61]. IL-10 mRNA expression is shown to be increased in peripheral blood mononuclear cells, pleural fluid and alveolar lavage fluid [62,63]. Increased IL-10 responses are also shown to be involved with increasing disease severity in patients infected with *M. tuberculosis* [64].

TGF- β : TGF- β produced by monocytes, dendritic cells and T regulatory cells also antagonizes protective immunity in TB [65]. It inhibits antigen production from macrophages and down regulates pro-inflammatory cytokines such as IFN- γ produced by T cells. TGF- β is found to be increased in TB [65,66]. It is also known to induce IL-10 and is shown to act in concert to inhibit Th1 mediated pro-inflammatory cytokine responses [67].

REGULATION OF IMMUNE RESPONSES BY *M. TUBERCULOSIS*

Cell mediated immune response play a central role in the immune defense against TB. However, there is a critical balance between induction of pro-inflammatory anti-pathogen responses and anti-inflammatory responses required to limit damage to host tissues that determines the outcome of TB infection [68]. Reports have shown that TB is associated with depressed pro-inflammatory cytokine profile, however higher expression of chemokines helps in recruitment of immune cells at the site of infection [69]. The impaired mechanisms of T cells in patients with TB suggest that T cells responses to *M. tuberculosis* might be subjected to regulatory mechanisms and these are believed to limit immune mediated tissue pathology [70]. Although Th1 responses help to limit bacterial replication, they are also associated with significant immune-pathology. Activation of cytokines can occur in response to a direct stimulation of cells by *M. tuberculosis* or through a 'bystander' effect whereby neighboring cells are regulated through cytokine produced by the directly stimulated cell [71].

Regulatory T cells were identified as another subset of T cells that maintain the immunological homeostasis and prevent immuno-pathology caused by exacerbated pro-inflammatory responses produced by Th1 cells. Regulatory T cells suppress Th1 mediated immune responses in such a way that sufficient immunity remains for clearing infectious agent [72]. Subsequent to activation of pro-inflammatory cytokines, a class of proteins called SOCS is up-regulated. As the name implies these are known to regulate the strength of cytokine signals. Cytokines stimulation result in the induction of SOCS molecules. These proteins inhibit signaling pathways that initially led to their production thus acting as a part of negative feedback loop [73].

CD4+ CD25+ FOXP3+ T REGULATORY CELLS

T regulatory cells (**Tregs**) are a subset of T cells that express CD4+ and CD25+ and the forkheadbox-P3 (**FOXP3**) transcription factor. The expression of FOXP3 modifies the phenotypic and functional properties of Tregs. Two main subsets of Tregs that have been described are: naturally occurring CD4+ CD25+Tregs (**nTregs**) and inducible Tregs (**iTregs**). The nTregs develop in the thymus and constitutively express the α -chain of the Interleukin-2 (**IL-2**) receptor (**CD25**) and the transcription factor FOXP3. While, iTregs develop in the periphery from CD4+ T cells upon stimulation with antigen. iTregs express CD25 and, in humans, FoxP3 [74,75].

Tregs cells are fundamental in avoiding detrimental effect of immunity to self, leading to autoimmunity as well as the damaging effect of disproportionate immune response. On the hand, Tregs have been reported to inhibit protective immune responses to pathogens; they secrete down-modulatory cytokines such as IL-10 and TGF- β and hence play an essential role in regulating both innate and adaptive arms of immune responses [75].

In the murine model of TB, previous studies have demonstrated that decreased Treg numbers are associated with lower mycobacterial load [76]. Previous work on *M. tuberculosis*-infected individuals suggests that CD4+ CD25+Tregs might contribute to chronic infection by preventing effective IFN- γ responses to *M. tuberculosis* stimulation [77]. This is further supported by the demonstration that infected patients have increased frequencies of CD4+ CD25+ FOXP3+ T cells in patients with active tuberculosis as compared with uninfected controls [72]. Patients with active TB are shown to have higher levels of CD4+CD25high+FOXP3+Tregs in blood when compared to latently infected individuals or uninfected controls [72]. Particularly, FOXP3-expressing CD4+ cells are elevated at the sites of infection such as granulomas and in pleural fluid of patients with active disease [78,79]. Recent studies show that suppressed IFN- γ responses to protective *M. tuberculosis* antigens such as, heparin-binding haemagglutinin adhesion (**HBHA**) can also be attributed to rise in FOXP3+Treg cells [80]. It has also been shown that Treg depleted and Th reconstituted cells can effectively control *M. tuberculosis* [81]. Additional studies have shown that anti-tuberculosis therapy decreases Treg cells and that Treg cells in recovered patients were significantly lower and the values were comparable with healthy donors [82].

While the role of Tregs in tuberculosis infections is increasingly more apparent, their role in disease dissemination in tuberculosis has not been studied.

SUPPRESSOR OF CYTOKINE SIGNALING MOLECULES

Classically, cytokine activity is characterized by rapid induction, short half-life and high local concentration. An essential component of cytokine transduction also includes a timely termination of signals. Many cytokines are known to exert their biological functions through the Janus kinases (**JAK**) and signal transducers and activators of transcription factors (**STAT**). SOCS, previously known as cytokine inducible SH2 containing protein molecules, belongs to a family of 8 proteins (**CIS, SOCS1-7**) that are known to function as a negative regulator of cytokine signaling. Of these SOCS1 and SOCS3 are widely studied [83]. SOCS proteins comprise of an amino terminal domain of variable length and sequence, a central SH2 domain, and a carboxy terminal 40 residue motifs known as the SOCS box. The SH2 domain of SOCS1 molecules is shown to directly bind to the activation loop of JAKs while the SH2 domain of SOCS3 molecules bind to the phosphorylated tyrosine residues on activated cytokine receptors through a kinase inhibitory region (**KIR**). KIR functions as a pseudo-substrate and is essential for the down-regulation of cytokine signals. The SOCS box interacts with ubiquitination machinery and plays a role in degradation of the protein [83].

SOCS1: SOCS1 is induced in response to multiple cytokines including IL-2, IL-3, IL-4, IL-6, Growth hormone (**GH**), prolactin, Eosinophil peroxidase (**Epo**), Leukemia inhibitory factor (**LIF**), IFN- γ , IFN- α , Oncostatin M (**OSM**), Thymic stromal lymphopoietin (**TSLP**), Thrombopoietin (**Tpo**) and Insulin-like growth factor-I (**IGF-1**) and is implicated in their feedback inhibition [83]. The physiological role of SOCS1 was investigated by target disruption of SOCS1 gene in murine models [84-86]. Mice deficient in SOCS1 showed stunted growth and died within 3 weeks due to aberrant T cell activation and macrophage infiltration of major organs [84]. The pathology associated with SOCS1 deficiency was a result of impaired IFN- γ signaling. These results were confirmed when SOCS1/Rag2 (Recombination activating gene 2) double knockout mice were found to survive much longer [87]. Increased levels of SOCS1 molecules have been demonstrated in lung cells of patients with active pulmonary tuberculosis [88]. Increased SOCS1 in lung macrophages also lead to decreased capacity to promote Th1 type immunity. Increase in SOCS1 has been shown to decrease macrophage activation by IFN- γ by inhibiting STAT-1 activation thereby permitting increased mycobacterial persistence [73]. Corresponding to these findings less than 30% of alveolar macrophages was found to express NOS2 supporting the fact that depressed anti-microbicidal activity is present in lungs during active tuberculosis [88]. Protection against infection with *M. tuberculosis* therefore is dependent on regulation of IFN- γ mediated responses via SOCS1. Studies have shown that *M. tuberculosis* cell wall component "Trehalose 6, 6'-dimycolate (**TDM; cord factor**) is a highly efficient stimulator of SOCS1 expression [89]. SOCS1 is also known to inhibit responses to IL-12, resulting in an impaired IFN- γ secretion and hampers intracellular mycobacterial control [90]. Increased expression of SOCS1 was also found to be associated with increasing disease severity in PTB [91].

SOCS3: SOCS3 another member of SOCS molecules is known to control the cellular responses to IL-2, IL-3, IL-4, IL-6, GH, prolactin, Epo, LIF, IFN- γ , IFN- α , CNTF, leptin, OSM, IGF-1 and insulin [83]. SOCS3 regulation of JAK/STAT signaling pathway is preferably known to function via inhibiting STAT3. Immune polarization via SOCS3 has an impact on disease susceptibility to TB [87]. Increased expression of SOCS3 in whole blood from TB patients has been previously described [92]. Mainly SOCS3 functions as a pro-inflammatory gene inhibiting IL-6/gp130 signaling in macrophages however, in pathologic situations SOCS3 suppresses pro-inflammatory responses [87]. SOCS3 is reported as one of the highly expressed protein in macrophages and is known to control the switch between classical (**M1**) and alternate activation of macrophages (**M2**) [93]. Furthermore loss of SOCS3 expression in macrophages is associated with reduced inflammation and a switch to alternate macrophage activation with increased secretion of IL-10 [93]. SOCS3 has been shown to inhibit STAT3 activation. STAT3 mediates IL-10 and TGF- β de novo synthesis and signaling in myeloid cells [94]. TGF- β also contains potential STAT3 binding site [95, 96]. IL-10 and TGF- β hamper TNF- α and IFN- γ responses in myeloid cells or the secretion of IFN- γ by T cells [97]. Secretion of IL-10 and TGF- β is enhanced in TB compared to un-infected controls [98]. Murine models deficient in SOCS3 expression are also reported with impaired immune responses due to the increased production of IL-10 and TGF- β [95]. STAT3 also negatively regulates nuclear factor- κ B pathway which is important in regulating immune responses against infection and SOCS3 negatively regulated this process [99,100].

A recent study has shown that SOCS3 expression is increased in T cells from TB compared with individuals with latent TB [101]. SOCS3 also inhibits IL-2 responses thereby interfering with T cell differentiation.

CONCLUSIONS

In summary, in immune competent individuals the abovementioned T cell mechanisms are responsible for effective clearance of *M. tuberculosis* infection. However, in the case of co-infections such as with Human immunodeficiency virus (**HIV**) or in the case of co-morbidities such as diabetes, host immunity becomes compromised and is less effective. Thus creating the opportunity for establishment of active *M. tuberculosis* infection where the bacillus modulates the host for its own survival and persistence.

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