Role of Myeloid-Derived Suppressor Cells and Regulatory T-Cells in the Tuberculous Granuloma

Laurene S. Cheung, Geetha Srikrishna, and William R. Bishai

Abstract In the course of *Mycobacterium tuberculosis* (*M.tb*) infection, while a robust immune response is required for containment and clearance of the pathogen, immune-mediated tissue damage may also occur. Immune suppressive cells, such as regulatory T cells (Tregs) and myeloid-derived suppressor cells (MDSCs) are recruited to the site of infection, but in the process of controlling immune responses can promote pathogen survival. Tregs are known to be elevated in tuberculosis (TB) patients with active disease and studies in animal models demonstrate that Tregs inhibit effector T cell function through multiple mechanisms during *M.tb* infection (Guyot-Revol et al., Am J Respir Crit Care Med 173:803–10, 2006). More recently, increased levels of MDSCs have been found in patients with active TB and although less is known about their role in infection, it has become clear that MDSCs are very effective in suppressing T cell responses in tumors (El Daker et al., PLoS One 10:e0123772, 2015). In this chapter, we will give a brief overview of the early immune response to *M.tb.* infection and the host's attempt to contain infection through the formation of granulomas in the lung. We will then review the function of MDSCs and Tregs and what is known about their role during TB infection. Finally, we will discuss currently available drugs that can target these cell populations and their potential use for the treatment of TB.

Keywords Tuberculosis · Regulatory T cells · Myeloid-derived suppressor cells · Granuloma · Host-directed therapy · Immune checkpoint inhibition · Effector T cells · Immune suppression · Immunotherapy · Inflammation

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Introduction

> Now, here, you see, it takes all the running you can do, to keep in the same place
> (Red Queen to Alice in Lewis Carroll's *Through the Looking-Glass*)

The Red Queen hypothesis, an evolutionary theory proposed by biologist Leigh Van Valen, suggests that reciprocal coevolution of hosts and pathogens selects for discrete molecular events that lead to continued survival of both. Tuberculosis (TB) is an ancient human disease, estimated to have originated and evolved for over many thousands of years alongside the modern human population [1]. This reciprocal coevolution has made *Mycobacterium tuberculosis* (*M.tb*) one of humanity's most successful obligate pathogens, with the mycobacterial niche so fine-tuned within this primordial host-pathogen relationship, that today it is estimated that one-fourth of the world's population harbors *M.tb* [2]. According to the CDC, there were 10.4 million incident cases of TB worldwide in 2015, associated with 1.8 million deaths, making TB one of the world's most significant medical challenges. The emergence of multi-drug-resistant and extensively-drug resistant (MDR and XDR) *M.tb* strains, combined with limited resources in developing communities, and the lack of quality diagnostics and effective chemotherapy regimens, critically hinder control of this disease. Failure to contain the disease globally has led to increased and targeted efforts towards identification of novel therapeutic targets. While many bacterial pathogens have devised multiple strategies to avoid phagocytic engulfment and killing, invading *M.tb* has adopted a highly successful strategy, wherein following recognition and phagocytosis by host pulmonary alveolar macrophages, the bacteria use these cells as sanctuary sites for persistence and propagation. Active or primary TB, characterized by symptomatic disease, is manifest only in a small percentage of infected individuals. In a majority of infected individuals however, latency is established even in the presence of a fully competent host immune system that helps to contain, but not eliminate, the infection, though the individual remains asympotmatic. Lifetime risk of reactivation or post-primary TB in individuals with latent infection (LTBI, Latent Tuberculosis Infection) is about 5–10%, with the risk being higher in immunosuppressed individuals, as seen with HIV co-infection or following treatment with immunosuppressive drugs. Bacillus Calmette–Guérin (BCG), the only available vaccine against TB for the last 90 years, has an efficacy ranging from 0–80% in adults. This variation has been attributed to geographical differences and pre-exposure to endemic mycobacteria among others [3]. The inadequacy of the vaccine to protect all young and adult populations has intensified global efforts to improve its efficacy and to develop newer vaccines.

A highly evolved and coordinated sequence of immune evasion strategies, involving both innate and adaptive immunity, allows *M.tb* to avoid immune-mediated clearance by the host [4]. For example, inside pulmonary alveolar macrophages, *M.tb* arrests phagosome maturation and modulates cell death pathways that allow replication in the early endosomal compartment [5]. Bacteria are sheltered and sequestered within organized lung structures called granulomas which consist of a dynamic population of immunologically altered macrophages and other cells of

myeloid and lymphoid origin [6–8]. Host adaptive immunity is triggered by the processing of mycobacterial antigens by antigen-presenting cells that activate T lymphocytes, and is further enhanced by pro- and anti-inflammatory cytokines released during infection. Multiple effects mediated by the cells within the granuloma, while limiting bacterial growth, also suppress immune responses, and provide a survival niche from which the bacteria may ultimately disseminate [6, 9]. The functional outcome of this dynamic cell recruitment by the host and manipulation of adaptive immunity by *M.tb* is a fine-tuning of the balance between pro- and anti-inflammatory networks [4, 10] that dictates the outcome of disease, akin to immune events of a tumor microenvironment. Understanding of this dynamic host-pathogen interaction is therefore of major importance in the development of novel host-directed therapies (HDTs) against TB, and to improve vaccine efficacy.

Strategies developed by *M.tb* for evading host defense include manipulation of the immune system towards immunosuppression and tolerance. Recent studies in patients and animal models show that among the host cell populations that promote immune evasion and suppression during *M.tb* infection are myeloid-derived suppressor cells (MDSC) and regulatory T-cells (Tregs). MDSCs exhibit immune-regulatory potential in both adaptive and innate immunity. They have been shown to accumulate in lungs during pulmonary TB, and they not only dampen anti-mycobacterial T-cell responses, but also phagocytose and shelter *M.tb* intracellularly. In addition, MDSCs promote the development of $CD4^{+}CD25^{+}FOXP3^{+}$ Tregs, which are known to play an important role in the prevention of autoimmunity and in the control of pro-inflammatory immune responses. Concomitant with increase in MDSC, the numbers of Tregs in the lung have also been shown to increase dramatically during *M.tb* infection in both animal models and in human patients. Skewing host immunity by selectively targeting cells that promote bacterial persistence and mediators that either promote their accumulation or lead to suppression of effector responses, might therefore prove highly valuable in reducing *M.tb* persistence and disease. This chapter focuses on the role played by MDSC and Tregs in TB pathogenesis and on possible new therapeutic avenues for targeting these cells in TB management.

The Tuberculous Granuloma: Host Immune Quarantine of *M. tb.*

Innate Immune Recognition of M.tb.

The lung serves as the predominant site of entry, containment, long-term persistence, disease manifestation, and ultimate spread of the pathogen. The most characteristic lesion of TB is the tuberculous granuloma (Fig. 1), which serves as an immune quarantine where the infection can be contained and controlled, but not altogether eliminated [8, 11]. After aerosol inhalation, the bacteria are deposited in

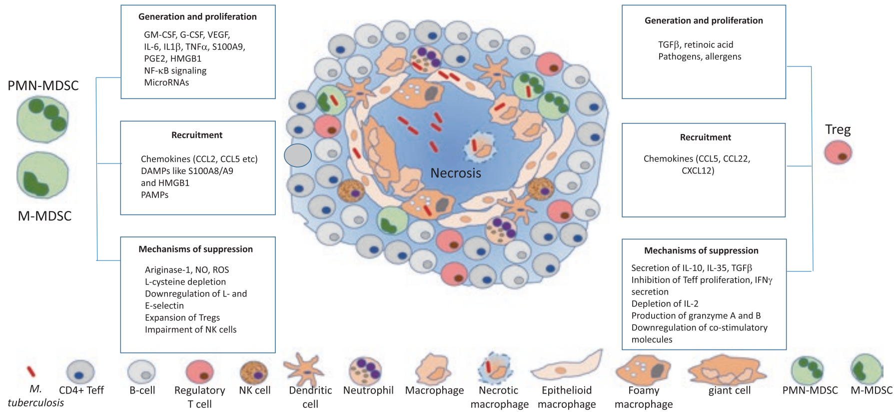

Fig. 1 Cellular composition of the necrotic granuloma: factors promoting immunosuppressive cells and their known mechanism of action. Upon *M.tb* infection, bacteria are phagocytosed by macrophages and recruitment of additional macrophages, neutrophils, dendritic cells, and lymphocytes leads to the formation of a granuloma in an attempt to contain the infection. In addition to alveolar macrophages, epithelioid macrophages, foamy macrophages, neutrophils, and myeloid derived suppresor cells (MDSCs) support intracellular replication of bacilli. Bacilli are found in these cell types as well as the central necrotic region of the granuloma. T cells, B cells, Tregs and MDSCs migrate to the granuloma as well. While Tregs and MDSCs may prevent destructive hyper-inflammation, these suppressive cells also inhibit T effector responses during TB infection, leading to pathogen persistence

the alveoli. An initial innate immune response ensues when *M.tb* is engulfed by alveolar and interstitial macrophages, as well as local dendritic cells (DCs), following recognition by pattern recognition receptors (PRRs) present on these cells [12]. Several classes of germline-encoded PRRs associated with myeloid cells, either on the cell surface or cytosolic, including Toll-like receptors such as TLR2 and TLR9, C-type lectin receptors, mannose receptor and scavenger receptors, NOD2 and NLRP3, members of the NOD-like receptor family, AIM-2 like receptors (absent in myeloma, ALRs), nucleic acid sensors such as cyclic GMP-AMP synthase (cGAS) and stimulator of IFN genes, recognize specific mycobacterial pathogen-associated molecular patterns (PAMPs) and promote innate immune responses including the activation of NF-κB, the Type I interferon (IFN) response, and inflammasome activation, collectively known as the cytosolic surveillance pathway (CSP) [13]. On human DCs, the major receptor involved in *M.tb* recognition appears to be DC-SIGN [13]. This innate immune response serves a critical role as the first line of defense against the invading pathogen. However, *M.tb* has evolved several mechanisms to survive within the hostile environment of the macrophage. Despite sequestration within a membrane-bound phagosome, *M.tb* components also gain access to the macrophage cytosol via the bacterial secretion system ESX [14]. This allows for host recognition of the *M.tb*-derived nucleic acids, dsDNA and c-di-AMP, triggering CSP and autophagy. Our recent studies for example, show that secreted

M.tb-derived c-di-AMP functions as a PAMP to activate the host CSP and autophagy during infection [15].

Type I interferons (IFNα/β) activated during *M.tb* infection of macrophages promote downstream signaling pathways leading to induction of a large number of IFN-stimulated genes (ISGs). Studies in mouse models of infection and human patients with active TB have now clearly established that Type I interferons promote the progression of disease [16, 17]. Patients with active TB have a prominent whole blood IFNα/β-inducible transcriptional signature that correlates with the extent of disease [18]. On the other hand, signaling pathways activated by *M.tb* infection of macrophages lead to the production of IL-1α and IL-1β and other key pro-inflammatory mediators TNFα, and IL-6, all of which stimulate vigorous anti-microbial responses [19]. There also appears to be a significant cross-talk between these pro- and anti-microbial responses. While IFNα/β suppress the production of host-protective cytokines, IL-1α and IL-1β inhibit IFNα/β induction. This counter-regulation of IL-1 and Type I IFN signaling appears to provide a balanced host response that helps to keep the infection contained [20, 21]. Additionally, in certain contexts, Type I IFNs may play a protective role during TB infection. Studies in mice demonstrate that in the absence of IFNγ signaling, loss of Type I IFN signaling led to increased lung bacterial burden and pathology, due to increased frequency of alternatively activated macrophages and impaired recruitment and differentiation of macrophages and myeloid DCs in the lungs of infected mice [22, 23].

Immune Cell Recruitment and Initiation of an Adaptive Immune Response to M.tb

Following *M.tb* infection, chemokines and many related mediators produced by activated macrophages lead to additional recruitment of monocytes, neutrophils and dendritic cells to the site of infection, leading to a focal accumulation of mononuclear cells [24]. This initial innate response is followed by initiation of adaptive immunity when dendritic cells that have phagocytosed *M.tb* migrate to lymph nodes and present antigen and principally prime CD4+ and CD8+ T-helper lymphocytes [24, 25]. The adaptive immune response to *M.tb* is delayed, however, relative to responses to other pathogens. Infected humans do not become tuberculin positive until six weeks after exposure, and mice infected with low dose *M.tb* show delayed T cell response (3–4 weeks) in comparison to responses to other acute bacterial and viral infections such as to *Listeria monocytogenes* and influenza virus, which typically peak between 7 and 10 days after infection [26]. This delay has been attributed in part to regulatory T-cells [27]. In *M.tb* infection, DC-derived IL-12 is essential for development of Th1 cells [28]. Primed *M.tb* antigen-specific CD4+ effector Th1-cells migrate to infection sites and promote strong anti-mycobacterial effects through the secretion of IFN-γ, TNF-α, and IL-2 [29]. Mice that lack IL-12 or IL-12 receptor, which show defective Th1 responses, are highly susceptible to *M.tb*

infection, as are individuals with mutations in IL-12 or its signaling components [30]. Antigen-primed CD8+ T cells also produce IFN-γ, and TNFα, although to a lesser extent than CD4+ T cells [31]. In addition, CD8+ T cells perform cytolytic functions capable of killing *M.tb* infected cells. Although evidence for a role of CD8+ T cells in control of *M.tb* infection in humans has not yet been well established, infection in humans is known to induce generation of both MHC Class I restricted classical CD8+ T cells as well as non-classically restricted CD8+ T cells, which include CD1-restricted and MHC I-related (MR1) restricted T cells, such as mucosal associated invariant T cells (MAIT) [32]. These cells are classified as innate lymphoid cells (ILCs), which function as early responders similar to innate immune cells but exhibit functional overlap with adaptive immune cells. Unlike adaptive lymphocytes, ILCs do not express antigen-specific receptors that have undergone somatic recombination and generally respond to cytokines and engagement of activating receptors on their cell surface. Other ILCs recruited to the site of infection include NKT cells and γδ T cells, and adaptive immune cells like B-lymphocytes [33–35]. γδ T cells secreting IL-17, and NKT cells expressing TCR and NK cell markers serve as intermediaries between the innate and adaptive immune responses [36]. In mice, Th17 cells promote neutrophil accumulation and tissue damage, but also promote recruitment of IFN-γ producing cells and granuloma formation [37, 38]. Th2 cells, which counter-regulate Th1 cells, likely impair protective immunity against TB, but this has not been fully established. Prolonged *M.tb*-induced TLR-2 signaling also promotes recruitment to the granulomas of $CD4^+$ $CD25^+$ $FoxP3^+$ regulatory T cells that dampen immune responses [39]. As discussed in detail later in this chapter, they are a major source of anti-inflammatory cytokine IL-10 and contribute to the down modulation of the immune response to the pathogen [6]. The infiltrating leukocytes ultimately remodel the infection site to form a granuloma, which helps to seal off the infection by creating a cellular and cytokine microenvironment of optimal immune response, but does not completely eradicate bacterial replication and persistence [40]. This is because *M.tb* continues to survive and proliferate in the mononuclear cells by (1) interfering with phagosome-lysosome fusion (2) inducing anti-inflammatory responses and (3) infecting newly recruited uninfected macrophages after exiting dying cells. Macrophages within granulomas have a high turnover rate and also demonstrate considerable phenotypic heterogeneity and functional plasticity [7]. Besides classically activated M1 macrophages, which inhibit *M.tb* replication, granulomas contain alternatively activated M2 macrophages, and transformed macrophages such as epithelioid, foamy, and multinucleated giant cells, which promote *M.tb* persistence by exhibiting anti-inflammatory phenotypes. Myeloid cell populations in TB granulomas has been recently expanded to include MDSCs, which are well known inhibitors of T-cell responses [7]. In addition, other recruited immune cells also aid in *M.tb* persistence and ultimate spread. Polymorphonuclear leukocytes (PMN) are recruited early to the lungs primarily in response to CXCL5 produced by pneumocytes and macrophages during *M.tb* infection [41]. Although they exhibit early anti-bacterial effects, and contribute to granuloma assembly [42], they secrete matrix metalloproteinases

that are important drivers of cavitation preceding release and spread of *M.tb* [43]. A study in CXCL5 deficient mice demonstrated that decreased PMN recruitment to the lungs during *M.tb* infection due to lack of CXCL5-mediated signaling led to enhanced survival [44].

TB Granuloma Morphology and Heterogeneity

Three distinct types of granulomas have been observed in human disease [8, 45, 46]. The early stage of disease is characterized by a solid non-necrotic granuloma with a central area containing *M.tb* infected macrophages, surrounded by non-infected macrophages, neutrophils and CD4+ and CD8+ T cells, B-cells, and further by a layer of fibroblasts, collagen and extracellular matrix components [47, 48]. In early stages, a pronounced pro-angiogenic vascular endothelial growth factor response leads to neovascularization promoting a dynamic influx of cells. Studies in *M.tb* infected macaques suggest that early granulomas can harbor bacterium that replicate rapidly followed by either bacterial killing and control or progression of the lesion [49]. In situations when the granulomatous immune response becomes ineffective, active post-primary TB disease ensues. The lungs of such individuals contain enlarging granulomas that differentiate with time. Extensive neutrophilic infiltrates lead to a central core of suppuration and necrosis, forming the necrotic granuloma that expands and causes tissue damage [50]. Liquefaction of dead immune cells in the core allows for the development of the caseous granuloma, which is highly permissive for bacterial replication. Matrix degradation and dysfunctional tissue remodeling cause fibrosis, leading to the development of cavitary pulmonary disease that allows access of *M.tb* into alveoli and outward spread [51]. Cavity formation involves breakdown of extracellular lung matrix by specific hydrolytic enzymes, including proteinases, nucleases, and lipases. Collagen fibrils, specifically type I, III, and IV collagen, are the major structural components of the human lung extracellular matrix. They are highly resistant to enzymatic breakdown and can only be degraded by specific matrix metalloproteinases which are activated by signaling pathways involving mitogen-activated protein (MAP) kinase, PI3-kinase/Akt pathway, and transcription factors NF-κB and AP-1 macrophage infection, [52–54]. Cavitation correlates with bacterial abundance in the sputum [55, 56]. TB transmission is therefore highly increased by cavitary disease. Clinically, cavitation impairs the efficacy of antibiotics, contributing to treatment failure and the emergence of antibiotic resistance [57, 58].

Although pulmonary TB is the most common presentation, *M.tb* can also disseminate into other organs causing extrapulmonary TB [59]. Frequent sites of extrapulmonary infection include the pleura, lymph nodes, bones and joints, meninges in the central nervous system, larynx, skeleton especially the spine, eyes, gastrointestinal and genitourinary tracts, adrenal gland, and skin. Dissemination from initial infection site indicates spread from an unprotected pulmonary granuloma or bacterial dissemination into the sites via regional lymph nodes.

Myeloid-Derived Suppressor Cells: Serving Up a Double Whammy in the Tuberculous Granuloma

MDSCs: Discovery and Characterization in Disease

While latent *M.tb* infection is characterized by a balance of *M.tb* specific cellular and cytokine immune responses as discussed above, development of active disease is known to correlate with impaired immune cell responses [60, 61]. Reduced polyfunctional IFN-γ^{+}IL-2^{+}TNFα^{+} CD4+ T cells, increased TNFα single-positive CD4+ T cells, progressive T cell dysfunction, and impaired proliferation of *M.tb*-specific CD4+ and CD8+ T cells correlating with high bacterial burden have been found in patients with smear-positive TB, relative to patients with smear-negative TB and latent TB [62]. However, the underlying mechanisms leading to impaired T-cell responses in active TB are not completely understood. Recent research has begun to focus attention on immune cells and mediators that actively promote bacterial growth by suppressing anti-mycobacterial immune responses in TB. Among the immune cells that face scrutiny are a heterogeneous group of suppressive myeloid cells, which have developed evolutionarily to prevent excessive tissue damage in the host during infections and to promote wound healing and tissue remodeling, but also found to be co-opted by tumors and pathogens to support immune evasion and growth in a suppressive niche amidst host immunosurveillance [63–65]. Interestingly, they were first identified in 1978 as a suppressive population activated in the spleens and bone marrow of mice after the administration of BCG [66]. Further studies of immune tolerance demonstrated a population of "natural suppressor" cells in the neonatal spleen [67]. Studies in the late 1990s and early in the last decade identified the cells to accumulate in lymphoid organs, blood, liver, lungs and tumors in many mouse models of cancers, and to be phenotypically similar to neutrophils and monocytes, but functionally distinct [68, 69]. The suppressor cells have now also been identified in many different human tumors, including head and neck cancers, gliomas, renal, prostate, pancreatic, hepatocellular and non-small lung carcinomas among others [70, 71]. Their phenotypic heterogeneity had rendered their characterization and terminology contentious, and in 2007, leading cancer investigators suggested naming the cells as myeloid-derived suppressor cells or MDSCs, a terminology that defines both their origin and their functional nature [72]. Their characterization has been revisited more recently [73]. Accumulating evidence of their clinical significance has rendered them an integral part of the field of tumor immunology and a promising target for cancer immunotherapy, as well as infectious diseases [74].

MDSC Markers and Mechanisms of Suppression

MDSCs are classified as monocytic or M-MDSCs, and polymorphonuclear or PMN-MDSCs [73]. In cancers more than 80% of MDSCs are PMN-MDSCs, but both populations induce host-driven T cell tolerance that promotes immune evasion,

thus limiting the efficacy of immune-based therapies. In mice, PMN-MDSCs are defined as $CD11b^{+}Ly6G^{+}Ly6C^{low}$, and M-MDSCs as $CD11b^{+}Ly6G^{-}Ly6C^{high}$ cells. In humans, MDSCs have been mostly identified in blood and tumors described as $CD11b^{+}CD33^{+}HLA\text{-}DR^{low/neg}$ cells. Human PMN-MDSCs are $CD14^{-}$ and $CD15^{+}$, while M-MDSCs are $CD14^{+}$ and $CD15^{-}$. Some of the $CD33^{+}HLA\text{-}DR^{low/neg}$ cells are myeloid progenitors (or early MDSCs). Newer markers that have been identified to characterize MDSCs include CD124 (IL-4Rα), CD40, CD80, CD115 and S100A9. These markers do not however, define any specific subpopulations, and since none of the markers are unique to MDSCs, their identification requires further evidence of immune-suppressive properties.

The most important functional characteristic of MDSCs is their ability to inhibit antigen-specific and non-specific activation and proliferation of $CD4^{+}$ and $CD8^{+}$ T cells. While PMNs and monocytes/macrophages are recruited early in an attempt to control infection by phagocytosing bacteria and facilitating activation of T cells [75], PMN-MDSCs and M-MDSCs directly inhibit T-cell-driven immune responses [65]. MDSCs utilize multiple mechanisms to inhibit T cells that include depletion of amino acids, generation of NO and reactive oxygen species (ROS), inhibition of T-cell migration, induction of Tregs and Th17 cells, and impairment of NK-cell mediated cytotoxicity [63, 76].

L-arginine is required for functional T-cell responses. MDSCs express arginase-1, which degrades L-arginine into urea and ornithine, thereby depleting it from the environment. MDSCs also express the inducible NO-synthase, which catalyzes the production of citrulline and NO from L-arginine, further contributing to L-arginine depletion [77]. Cysteine, an essential amino acid required for T cell activation, is normally obtained from cystine exported from macrophages because T cells lack cystathionase, which converts methionine to cysteine. By sequestering cystine, MDSCs limit its availability for T-cell proliferation [78]. NO also inhibits JAK3, STAT5, ERK, and AKT, blocking IL-2R mediated signaling pathways, thereby impairing the generation of effector and memory T cells [79]. In addition, MDSCs generate reactive oxygen species (ROS) through isoforms of superoxide-generating NADPH oxidase [80], which disrupt the T-cell function by modifying its TCR-ζ-chain [81]. The nature of suppression depends on the subpopulation of MDSC. While M-MDSCs mainly generate NO, PMN-MDSCs produce higher levels of ROS [82]. MDSCs also prevent the homing of T cells to draining lymph nodes and tumor sites by downregulating L-selectin on naïve T cells and E-selectin on vasculature [83, 84].

MDSCs have been shown to induce the expansion and activation of Tregs [85–87]. Expansion of Tregs is mediated through secretion of IL-10 and TGFβ, and requires the expression of CD40 on MDSCs [85, 88, 89]. More recently, it has been shown that MDSCs induce Th17 ($CD4^{+}$ $ROR\gamma t^{+}$ $IL\text{-}17^{+}$) cells through secreted IL-6 and TGFβ [90]. In addition, IFNγ and TNFα can promote survival and accumulation of MDSCs and MDSCs treated with these cytokines have been found to produce CCL4, a Th17 chemoattractant that facilitates recruitment of Th17 cells [80, 91, 92]. IL-17, in turn, has been found to activate the ERK1/2 pathway in MDSCs and promote survival and accumulation of MDSCs in animal models of cancer [93, 94].

MDSCs have also been shown to impair the function of NK cells. TGFβ and H_2O_2 produced by MDSCs decrease the expression of NK cell activating receptors NKG2D, NKp46, and NKp44 [95, 96]. MDSCs also decrease the ability of NK cells to induce apoptosis of target cells by down-regulating their production of perforin and by limiting their response to growth factor IL-2 [97].

MDSCs are normally present at low or undetectable levels in healthy individuals, but their numbers dramatically increase in chronic inflammatory states, likely as a compensatory mechanism to limit bystander tissue damage, and in cancers [70]. Tumors appear to have co-opted the cells to facilitate immune escape and consequently their growth and dissemination and initial studies in animal models suggest *M.tb* and other bacteria do the same.

Induction of MDSCs and Their Involvement in Disease

During tumor progression, MDSC are generated from a common myeloid progenitor, promoted by tumor-derived GM-CSF, G-CSF, VEGF, IL-6, IL1β and TNFα, prostaglandin E2 and damage-associated molecular pattern (DAMP) molecules S100A8/A9 [98]. A two-step model was proposed to describe their generation: inhibition of terminal differentiation of progenitors, and conversion of immature myeloid cells to MDSC. A major transcription factor involved in growth factor and cytokine-mediated MDSC expansion is signal transducer and activator of transcription STAT3. Activated STAT3 also induces the expression of S100A8 and S100A9, which block differentiation of immature myeloid cells into dendritic cells and macrophages and promote MDSC expansion [99, 100]. More recently, another DAMP molecule, HMGB1, has been shown to promote the expansion of MDSCs from bone marrow progenitor cells in vitro. Neutralization of HMGB1 in tumor-bearing mice reduces MDSC levels in the tumor, spleen, and blood [101]. In addition, MDSC themselves secrete pro-inflammatory mediators IL-6 and S100A8/A9, which leads to an autocrine feedback loop that amplifies MDSC accumulation in the tumor microenvironment [100]. Other related transcription factors of the STAT family, STAT1 and STAT6, activated by Th1 cytokine IFN-γ and Th2 cytokines IL-4 and IL-13 respectively, have been implicated in MDSC activation and function [102, 103]. Activation of NF-κB, which is downstream of TLR, IL-1R and TNFR signaling is known to lead to MDSC expansion, as do RAS and PI3K/Akt signaling [104].

MicroRNAs (miRNAs), endogenous ~22 nt long non-coding RNAs, which have significantly advanced our understanding of gene regulation, modulate host immune responses by regulating the expression of important genes involved in the differentiation of immune cells. Several miRNAs have been implicated in the accumulation and function of MDSC [105], but discrepancies exist. For example, miR-155 has been shown to both promote and negatively regulate accumulation of MDSC [106, 107]. In this regard, it is interesting to note that infection of human macrophages

with *M.tb* decreased the expression of miR-155 [108], and that miR-155 is significantly reduced in the serum of TB patients [109].

Chronic inflammatory states occur during TB infection and also precede tumor development. Many important cytokines, myeloid differentiation factors, as well as DAMP molecules such as S100A8/A9 and HMGB1, all of which promote MDSC expansion in tumors as described above, are also elevated during infections and acute and chronic inflammation. MDSCs have in fact been implicated in immune regulation of chronic inflammation, in asthma, and in autoimmune diseases such as autoimmune enterocolitis and encephalomyelitis [110, 111]. Microbial factors and PAMPs also induce MDSCs. For example, *Pseudomonas aeruginosa* induces MDSC generation through flagellin [112]. An increase in MDSC frequency has been observed in *H. pylori* infected mice and humans [113]. Fungal infections (*Aspergillus fumigatus* and *Candida albicans*) induce a subset of MDSCs through pattern recognition receptor Dectin-1 [114]. Cystic fibrosis patients with chronic *P. aeruginosa* infections demonstrate a higher MDSC frequency in blood compared to patients without *P. aeruginosa* infections or healthy control subjects [112]. Mice infected with *K. pneumoniae* show MDSC expansion and increased levels of IL-10 [115]. Immunosuppressive subsets of MDSC have also been demonstrated in *S. aureus* skin infection models [116]. Elevated levels of MDSCs are found in the serum of patients with sepsis, although in sepsis they can be both pro-inflammatory and immunosuppressive [117]. Such paradoxical dual roles are evident in the early and late phases of infections. Since many of the factors leading to MDSC expansion are elevated during host immune response to *M.tb* and formation of the granuloma, it seems only logical to speculate that MDSCs would also accumulate in TB, and may play a pathophysiological role.

Evidence for MDSC Involvement During TB Infection

In support of this hypothesis, Gr-1 (Ly-6G/C) positive cells, which appear to modulate T cell expansion through production of NO and superoxide anion, were identified in the spleen mice primed with heat-killed Mycobacterium [118]. BCG vaccination of mice was found to increase the levels of myeloid cells, which impaired T cell priming in the draining lymph node in a MyD88-dependent manner [119]. Subsequently, MDSCs were found to be induced not only in patients with TB, but also in individuals recently exposed to *M.tb* (household exposure) [120]. These cells inhibited activation and proliferation of CD4+ and CD8+ T cells, altered T-cell trafficking, and were associated with increased production of IL-1β, IL-6, IL-8, G-CSF, and MCP-1. Another study showed that in patients with active TB, the frequency of $CD244^{high}$ cells with MDSC phenotypes were significantly higher, and negatively correlated with the activation and function of $CD4^+$ and $CD8^+$ T-cells [121]. MDSC accumulation has also been observed in both lungs and blood of patients with active TB and anti-TB therapy appeared to reduce MDSC accumulation in the blood [122]. In mouse models of TB, accumulation of MDSC was found to correlate with

increased TB lethality [123]. Knaul et al. showed that MDSCs expressing *Arg-1* and *Nos2*, comprising a heterogeneous population of PMN-MDSC and M-MDSC with immunosuppressive properties and elevated IL-4R expression, accumulated in the lungs at the site of infection. Excessive MDSC accumulation in lungs correlated with increased TB lethality [124]. Treatment with all-trans-retinoic acid (ATRA), which is known to reduce MDSC in vivo, decreased the frequency of lung $CD11b^{+}Gr1^{+}$ MDSCs in *M.tb* infected mice. ATRA treatment significantly reduced bacterial loads and ameliorated pathology suggesting that MDSCs are potential targets for host-directed therapies [124]. In addition, the study demonstrated the presence of *M.tb* in purified MDSC subsets, strongly indicating that the role of MDSCs in pathogenesis is multifactorial; that in addition to providing immune suppression and evasion from host immune responses, they also phagocytose and harbor *M.tb*, thus offering a newly defined physical niche for *M.tb* survival within the lungs.

S100A8/A9 are secreted by MDSCs, and they promote differentiation of myeloid progenitors into MDSC in the bone marrow as well as autocrine accumulation and activation of MDSCs in tumors [99, 100]. They are calcium-binding protein molecules that are constitutively expressed by myeloid cells, and contribute to intracellular homeostatic processes. However, during infections, inflammation, and in tumors, they are also secreted into the extracellular medium, and serve as endogenous danger signals or DAMP, promoting immune responses and repair mechanisms through binding to cell surface receptors such as the Receptor for Advanced Glycation End Products (RAGE) and Toll-like Receptor 4 (TLR4) [125, 126]. It has been shown that in human patients with active TB and in nonhuman primate models and mouse models of *M.tb* infection, myeloid cells producing S100A8/A9 proteins dominate within the inflammatory lung granulomas and exacerbate inflammation [127]. In fact, recent proteomic studies suggest that S100A9 could serve a serum diagnostic biomarker for pulmonary TB and to discriminate pulmonary TB from other lung diseases such as pneumonia and lung cancer [128]. In BCG-challenged guinea pig lungs, administration of tasquinimod, which binds to S100A9 and blocks its interaction with cell surface receptors, impaired the formation of granulomas indicating that S100A9 plays an important role in the organization of the tuberculous granuloma [129]. All the above studies suggest that MDSCs may play a pathological role in the progression of TB and could therefore be targeted for anti-mycobacterial therapy.

Regulatory T Cells: Amplifying the Immunosuppressive Microenvironment Within the Tuberculous Granuloma

Tregs: Essential Mediators of Immune Homeostasis

As mentioned earlier, *M.tb* elicits both innate and adaptive immune responses in the host. While MDSCs straddle both the innate and adaptive systems in the immune hierarchy, another set of immunosuppressive cells found in the tuberculous

granuloma, the regulatory T-cells or Tregs, serve as an integral part of the adaptive immune system. Tregs were initially discovered as a specialized subset of CD4+ T cells expressing IL-2 receptor a chain (CD25), that play a pivotal role in maintaining self-tolerance and preventing autoimmune diseases [130]. They provide tolerance to both self-antigens and to commensal flora and innocuous environmental antigens and allergens [131]. Tregs express a specific marker, the transcription factor forkhead box P3 (FOXP3), which regulates expression of genes responsible both for the differentiation of Tregs, and for the suppression of immune response [132, 133]. In humans, FOXP3 deficiency results in the development of a multi-organ lymphoproliferative autoimmune disease, also known as immune dysregulation, polyendocrinopathy, enteropathy and X-linked (IPEX) syndrome [134, 135]. Tregs play a critical role in the induction and maintenance of peripheral tolerance in allogeneic stem cell transplantation [136]. Studies using graft-versus-host disease (GvHD) model systems have shown the adoptive transfer of Tregs inhibits the allogeneic immune response [137, 138]. In the periphery, it is now well established that Tregs also regulate host immune responses to pathogens by preventing uncontrolled immunopathology and collateral tissue damage associated with hyper-inflammatory reactions [139]. MDSCs have been shown to promote the expansion of Tregs, thus helping to maintain and amplify an immune tolerant microenvironment [85–87]. As with MDSCs however, Tregs can also be co-opted by pathogens such as *M.tb* to subvert effector immune responses, allowing their survival in the host [140, 141].

Based on their origin and function, two major populations of Tregs have been described: natural (nTreg) and induced (iTreg) cells. It has also been recently recommended that Treg populations be denoted by place of induction: “thymus derived” (tTregs) or “peripherally derived” (pTregs) [142]. nTregs or tTregs arising in the thymus form the major population of $CD4^+FOXP3^+$ Tregs, and they mediate tolerance to self-antigens [143]. iTregs ($CD4^+FOXP3^+$) or pTregs arise in peripheral lymphoid tissues from naive conventional $CD4^+FOXP3^-$ T cells after exposure to antigens. They are especially abundant in the gastrointestinal tract and in lungs and exhibit specificities against microbial antigens or environmental allergens, and also restrain immune responses to exogenous pathogens [144]. Their induction from naive $CD4^+$ T cells requires TGF-β and retinoic acid that are secreted by dendritic cells and resident macrophages [145]. Specific markers on human Tregs are lacking and therefore are defined by multiple regulatory markers and/or by demonstrating suppressive activity.

Mechanisms of Treg-Mediated Suppression of Immune Responses

Tregs produce immunosuppressive cytokines IL-10, TGFβ and IL-35 [146–148]. IL-35 is a recently identified member of the IL-12 family of heterodimeric cytokines. However, unlike other members of the IL-12 family, IL-35 is not produced by

antigen-presenting cells, but primarily by Tregs and B-regulatory cells [149]. IL-35 can directly suppress the proliferation and function of effector T-cells and increasingly being considered as a key mediator of immune suppression.

It is now recognized that Tregs are in fact heterogeneous and include several subpopulations that are able to suppress immune reactions, maintain self-tolerance and restore homeostasis after immune response. Recently, a unique population of iTregs expressing the marker CD39 has been identified. $CD8^+CD25^+FOXP3^+$ T cells and $CD8^+CD25^+CD39^+FOXP3^+$ T cells, both referred to as $CD8^+$ Tregs, are a small subset of an immunosuppressive population of $CD8^+$ suppressor T cells, which have been identified in *M.tb* and *M.bovis* infections [150]. IL-35 further promotes tolerance to infections by generating a potent population of IL-35-producing inducible Tregs called iTr35, which in turn produce IL-35, but not IL-10 or TGFβ [149]. $CD4^+$ type 1 regulatory T (T_R1) cells represent another subset of Treg cells defined by the expression of IL-10, a master regulator of inflammation, but do not express FOXP3 and CD25 [151]. The T_R1 cells express a number of transcription factors, such as c-MAF and IRF1, which are common to other T-cell populations.

Tregs suppress both innate immune responses, as well as induction of T-cell responses at the activation, proliferation, and differentiation stages and at the effector stages in tissues. Their suppressive activity targets dendritic cells, macrophages, NK cells, CD4+ and CD8+ T-cells, B-cells, and NKT cells and are mediated primarily through the secretion of inhibitory cytokines IL-10, TGFβ and IL-35 [152]. These cytokines directly inhibit effector T-cell (Teff) proliferation, and also the expression of MHC class II and co-stimulatory molecules on antigen-presenting cells, thus indirectly suppressing Teff activation. IL-2 is a major trophic cytokine for various T-cell subsets and expression of IL-2Rα chain (CD25) on the surface of Tregs allows them to bind and deplete IL-2 from the environment, which leads to inhibition of proliferation and apoptosis of effector T-cells [152]. Tregs also release granzymes A and B, which promote T-cell cytolysis. Granzyme A-induced cytolysis is perforin-dependent, FAS-FASL-independent, and requires cell-contact [153]. Treg cells derived from the tumor environment induce NK and CD8+ T cell death in a granzyme B- and perforin-dependent fashion [154]. Activated Tregs also express galectin-1 on the surface, which binds to relevant carbohydrate ligands on effector T-cells, inhibiting their proliferation and decreasing the production of IFNγ [155]. Tregs also function through down-modulation of antigen-presenting cells through interactions of CTLA-4-CD80/CD86 and LAG-3-MHC class II interactions [156, 157]. CTLA-4 is a Foxp3-dependent protein expressed on Tregs. Mice with Tregs that lack CTLA-4 protein expression develop lethal autoimmunity [156]. CTLA-4 on Tregs binds to costimulatory molecules CD80 and CD86 on dendritic cells reducing their availability for naive T-cells and hindering co-stimulation during antigen presentation. This is followed by development of anergy and apoptosis in antigen-specific T cells. Indoleamine 2,3-dioxygenase (IDO), induced by CTLA-4, depletes tryptophan in local tissue microenvironment, leading to reduced proliferation and apoptosis of Teff cells [158]. Recently, lymphocyte-activation gene 3 (LAG-3), homologous to CD4, has emerged as another important molecule that regulates T cell function [157]. It is expressed on different subsets of T cells and

also on B cells, NK cells and plasmacytoid DC. Lag-3 binds to MHC class II with high affinity, and negatively regulates cellular proliferation, activation, and homeostasis of T cells.

Role of Treg Suppression in Disease

A wide range of human pathologies has been associated with altered Treg function. These include pathologies associated with loss of tolerance, such as in immune dysregulation of genetic origin leading to autoimmunity, and allergic responses to food and environmental allergens [159, 160]. Mutations of *FOXP3* lead to the development of dysfunctional thymic Treg cells resulting in severe autoimmunity in the early-onset and life-threatening IPEX syndrome, manifesting with severe eczema, intractable diarrhea, and type I diabetes in the first months of life [134, 135]. Tregs have been implicated in suppressing effector responses in other autoimmune disorders such as myasthenia gravis and rheumatoid arthritis [159].

Tumors and pathogens exploit Tregs to suppress host immune responses for proliferation and survival. Tregs suppress anti-tumor immune responses, and along with MDSCs, contribute to the development of an immunosuppressive tumor microenvironment that facilitates immune evasion and cancer progression. An accumulation of FOXP3$^+$ Tregs is associated with unfavorable prognoses in many human cancers, including ovarian, pancreatic, lung cancers, and other malignancies [161, 162]. Tregs are also induced by a wide range of viral, bacterial and parasitic pathogens and play a dual role during infections: during acute infections, they benefit the host by limiting immune-mediated pathology and excessive inflammation. However, in the long-term, they also promote chronic pathogen persistence [140, 141]. Studies in mouse models of persistent *Salmonella enterica* infections show that after acute infection Tregs are elevated, but failure to completely eradicate the pathogen leads to a carrier state of persistent asymptomatic infection. Several studies have shown that *Helicobacter pylori* induce Tregs as well. Pathogen persistence during chronic *H. pylori* infection leads to chronic inflammation and gastric tumor induction. The role of Tregs has also been extensively studied in *M.tb* infections both in the latent and active disease states.

Treg Suppression of Immune Responses During TB Infection

Being an efficient manipulator of host immunity, *M.tb* elicits expansion of Tregs to support its persistence. Studies in mouse models show that Tregs act as checkpoint in three stages of *M.tb* infection: blocking effector cell responses in the lung, inhibiting priming and differentiation of T cells in the lymph node and inhibiting migration of activated T cells to the lung. In mouse models of *M.tb* infection, Tregs were found in granulomas in the lung, and were shown to prevent pathogen clearance

[163, 164]. Pathogen-specific Tregs induced by *M.tb* delayed priming of $CD4^+$ and $CD8^+$ T-cells in the pulmonary lymph nodes, thereby delaying migration of these cells to the lung [27]. Delayed onset of adaptive immunity allows initial establishment of infection. Depletion of $CD25^+$ cells early after *M.tb* infection decreased bacterial load and granuloma formation [165]. In macaques, an increasing frequency of Tregs in lungs and blood was found in animals developing active disease after challenge with *M.tb*. Tregs and IFNγ-producing effector T-cells expanded early after pulmonary TB infection, yet in vivo depletion of both T-effector cells and Tregs led to decreased resistance against granuloma progression [150]. These studies in animal models showed that Tregs aggravated the pathology of tuberculosis by blunting Th1 responses and thereby inhibiting *M.tb* clearance.

Treg cells are known to accumulate in human TB. Increased frequencies of $CD4^+CD25^{+/HI}$ cells and $CD4^+CD25^{HI}CD39^+$ cells have been identified in the peripheral blood and bronchoalvelolar lavage fluids in TB patients compared to healthy controls [166–170]. Frequencies of Tregs and levels of TGFβ have also been shown to be significantly higher in cavitary TB patients than in non-cavitary TB patients [171]. Circulatory $CD4^+CD25^+$ and $CD4^+CD25^+$ $FoxP3^+$ Treg cells were elevated in patients with cavitary MDR-TB and decreased after pulmonary resection [172]. The frequencies of Tregs in blood of TB patients declined after successful chemotherapy, but remained high in patients with emerging drug-resistant TB [173].

Increased frequencies of $CD4^+Foxp3^+CD25^+$ Tregs have also been found in peripheral blood and in broncho-alveolar lavage fluid in patients with active TB compared to individuals with latent disease [174, 175]. *M.tb* is known to migrate to and establish infection in extra-pulmonary sites. Treg cell frequency is higher in pleural fluid than in circulation in tuberculous pleurisy [176]. Patients with miliary TB also show increased frequencies of Tregs in peripheral blood, pleural fluid and bronchoalveolar lavage [177].

The presence of Tregs through granuloma evolution and increased frequency in cavitary disease suggests that they could play important roles in dissemination of TB. Increased frequencies during active disease compared to latent stages, and decline after therapy also indicate that functional signatures of Tregs can serve as biomarkers for disease progression and response to therapy.

Therapeutic Targeting of MDSCs and Tregs in TB

Studies from animal models of TB show that blocking the recruitment or functions of MDSC and Tregs impede progress of disease, suggesting that they can serve as valid targets for HDT (Table 1). Pre-exposure to mycobacterial antigens in endemic regions is believed to induce immune-regulatory cells in the host, which are further stimulated by BCG vaccination, and could partly account for the reduction in vaccine efficacy. Induction of Tregs has in fact been demonstrated in several TB-vaccine candidate trials [141]. Preventing induction of MDSCs and Tregs are therefore powerful new approaches to improving the efficacy of BCG and other novel anti-TB

Table 1 Therapeutic targeting of MDSCs and Tregs

Target cell effect	Therapy	Mechanism of action	Treatment outcome	Reference
Treg depletion	Anti-CD25	Antibody mediated depletion of CD25+ cells	Treg depletion in *M.tb* infected mice led to transient decrease in lung bacterial load	[165]
	Denileukin diftitox	Recombinant diphtheria fusion toxin targeting IL-2 receptor bearing cells	Treg depletion in *M.tb* infected mice resulted in decreased bacterial burden in lung and spleen	[178]
	Anti CTLA-4, ipilimumab	Antibody mediated depletion of CTLA-4+ cells	Treatment resulted in lower frequency of Tregs in tumors of bladder cancer patients. Treatment of BCG infected mice does not decrease bacterial burden	[179, 180]
Inhibition of Treg suppressive activity	Anti PD-1	Antibody blockade of PD-1	PD-1 blockade of Tregs from TB patients decreases suppressive activity in vitro	[173]
MDSC depletion	Sunitinib, sorafenib	Receptor tyrosine kinase inhibitor	Sunitinib treatment depleted MDSCs in a murine model of breast cancer	[181]
	Gemcitabine	Nucleoside analog	Gemcitabine treatment depleted G-MDSCs in patients with pancreatic cancer	[182, 183]
Promotion of MDSC differentiation	ATRA	Binds RAR nuclear receptors	ATRA treatment leads to decreased lung bacterial burden in *M.tb* infected mice	[124]
Inhibition of MDSC infiltration	Tasquinimod	Binds S100A9 and blocks interaction with its receptors	Tasquinimod treatment reduces infiltration of MDSCs into tumors in a murine model of breast cancer. Treatment of BCG-infected guinea pigs decreases lung granuloma formation	[129, 184, 185]
Inhibition of MDSC suppressive activity and reduction in MDSC numbers	Sildenafil, tadalafil, vardenafil	PDE-5 inhibitors	Tadalafil treatment lowers MDSCs in patients with head and neck cancer	[186, 187]

vaccines [188]. Vast literature on therapeutic targeting of MDSCs and Tregs in tumors show that they can be targeted by interfering with either their production, or blocking their trafficking to sites of infection, or inhibiting their immunosuppressive function.

Among major factors regulating MDSC generation in the bone marrow are stem cell factor receptor c-kit, and its downstream effector signaling that involves STAT3. Blocking of c-kit and STAT3 signaling using tyrosine-kinase inhibitors such as sunitinib and sorafenib have been shown to effectively reduce MDSC populations in both tumor-bearing mice and cancer patients [181, 189]. Gemcitabine, a nucleoside analog, is used as a chemotherapeutic in many cancers. Gemcitabine administration depletes MDSCs from spleens and tumors of tumor-bearing mice [182]. It has been shown to reduce MDSC and Tregs in patients with pancreatic cancer [183]. Since MDSCs are immature myeloid cells, an attractive therapeutic approach would also be to promote differentiation of MDSCs toward fully mature myeloid cells. Such an effect can be achieved by using ATRA (all-trans-retinoic acid). Treatment with ATRA substantially decreases the presence of MDSC in spleens of tumor-bearing mice and in peripheral blood of patients with renal cell carcinoma [190, 191]. In fact, in a rat model of *M.tb* infection, retinoic acid administration has been shown to reduce disease pathology and promote expression of TNFα and IL1β in alveolar macrophages [192]. Treatment with ATRA decreased the frequency of lung MDSCs, reduced bacterial loads and pathology in a mouse model of TB [124].

Another drug that targets MDSCs is tasquinimod, a second generation quinoline-3-carboxamide analogue. This analogue has shown anti-angiogenic, antitumor and immune-modulatory properties in preclinical models of prostate cancer and other solid tumors [193, 194] and has been found to inhibit the accumulation of immunosuppressive MDSC in tumors and premetastatic niches [193, 195]. Quinoline-3-caboxamides show high affinity binding to S100A9 protein [196]. Tasquinimod binds S100A9 protein on MDSCs and inhibits its interaction with cell surface receptors TLR4 and RAGE, thus reducing the infiltration of MDSC. Large randomized phase II trials of tasquinimod in men with chemotherapy-naïve metastatic castration-resistant prostate cancer (mCRPC) has demonstrated a significant prolongation in radiographic and symptomatic progression-free survival compared with placebo [184, 185]. Incidentally, in BCG-challenged guinea pig lungs, tasquinimod impairs the formation of granulomas, the organization of which is regulated by S100A9 [129].

The immunosuppressive function of MDSCs depends on their production of NO and ARG-1. Phosphodiesterase-5 (PDE5) inhibitors such as sildenafil, tadalafil, and vardenafil that are currently in clinical use, down-regulate ARG-1 and NO production. Preclinical studies in tumor models have shown that phosphodiesterase-5 (PDE5) inhibition is able to not only reverse MDSC suppression, but also Treg accumulation, thereby promoting antitumor immunity [86, 197]. In patients with head and neck squamous cell carcinoma (HNSCC) tadalafil administration has been shown to lower MDSCs and Tregs and increase tumor-specific $CD8^+$ T cells in a dose-dependent fashion [186, 187]. It has to be noted that human PDE inhibitors have emerged as an attractive strategy for adjunctive HDTs against TB. We have

found that in mouse models, addition of the FDA-approved cAMP phosphodiesterase inhibitors cilostazol (Type III PDE-I) as an adjunctive drug, either alone or with sildenafil (Type V PDE-I) to the standard TB treatment regimen, reduces tissue pathology, leads to faster bacterial clearance and shortens the time to lung sterilization by one month, compared to standard treatment alone [198].

Recently FDA-approved immune checkpoint inhibitors (ICI) for cancer treatment aim to re-establish anti-tumor immune responses by blocking inhibitory immune checkpoint molecules or their ligands, thereby enhancing Teff and cytotoxic T-cell (CTL) functionality. As mentioned earlier, Tregs highly upregulate expression of various immune checkpoint molecules (CTLA-4, PD-1, LAG-3), making them attractive targets for ICI. Monoclonal antibodies (mAbs) against CTLA-4 (ipilimumab) and PD-1 (nivolumab/pembrolizumab) have been used for the treatment of metastatic melanoma, non-small-cell lung cancer, advanced renal carcinoma and Hodgkin's lymphoma. Pre-clinical murine models have shown that anti-CTLA-4 mAbs activate Teff and CTLs, and promote ADCC (antibody-dependent cell-mediated cytotoxicity)-mediated depletion of intra-tumoral Tregs [199]. Intra-tumoral FoxP3^{+} Tregs were depleted by ADCC-mediated lysis following ipilimumab treatment in metastatic lesions of melanoma patients, although this observation remains controversial [179]. CTLA-4 blockade has not been thoroughly explored in the context of *M.tb* infection. BCG-infected mice treated with anti-CTLA-4 had increased lymphocyte recruitment to the lungs, however bacterial burden remained unchanged [180]. Further work must be done to determine whether CTLA-4 blockade changes Treg frequency or function and can alter the adaptive immune response to infection. Studies examining the effect of PD-1 blockade for treatment of *M.tb* have produced mixed results. PD-1 is highly upregulated on "exhausted" T cells, and inhibits T cell proliferation and IFN-γ and IL-2 production [200]. Preclinical studies show that nivolumab impairs Treg suppressive activity, possibly by downregulating intracellular expression of FoxP3, and promotes CTL proliferation [201]. Blockade of PD-1 on Tregs from patients with pulmonary TB decreased their suppressive activity in vitro, however loss of PD-1 in mice leads to increased susceptibility to *M.tb* [173, 202]. These data suggest that the effects of PD-1 blockade on immune response to infection are likely to be target cell-dependent.

Ontak® (denileukin diftitox; DAB389IL-2), is a FDA-approved biologic that specifically targets cells expressing the high affinity IL-2 receptor. It is a fusion protein comprised of the diphtheria toxin catalytic- and transmembrane domains fused to human IL-2 [203]. The cytotoxic potency of diphtheria toxin is selectively targeted to those eukaryotic cells that display high-affinity receptor for IL-2 [204–207]. The relative sensitivity of a given IL-2 receptor-positive cell line to Ontak® is dependent upon the pattern of expression of each of the three subunits of IL-2R. The high-affinity receptor (αβγ chains) is found on Tregs and activated T cells, while the intermediate affinity receptor (βγ chains) is found on resting memory T cells and NK cells. Expression of the α chain, also known as CD25, is used to identify Tregs expressing high-affinity IL-2 receptor. In 1999, Ontak® was approved by the FDA under the accelerated program for the treatment of refractory cutaneous T cell lymphoma

CTCL, based on durable objective responses [208], and used off-label for patients presenting with chronic lymphocytic leukemia, non-Hodgkin's lymphoma, and human T cell lymphotropic virus-1 [209]. The drug has also been used off-label to successfully treat steroid-resistant graft-versus-host disease [210], methotrexate-resistant psoriasis [211] and as an immunotherapeutic agent for the transient depletion of Tregs in patients with unresectable stage IV malignant melanoma [212, 213]. Ontak® treatment of *M.tb* infected mice resulted in decreased Treg frequency and bacterial burden in the lungs [178]. In addition, another Ontak®-related fusion protein DAB389mIL-4 is selectively toxic for eukaryotic cells that display the IL-4R on their cell surface, and the cytotoxic potency of this fusion protein toxin is directly proportional to the number of IL-4Rs on the cell surface [214]. Both fusion proteins therefore provide a unique and novel opportunity for the development of a new HDT for TB by targeting CD25$^+$ Tregs and IL-4R$^+$ MDSC which have engulfed *M.tb*.

Although current therapies for targeting MDSCs and Tregs have proven to be promising, there are impediments that need to be considered. Specifically, the heterogeneity of MDSC and Tregs highlights the need for specific markers to be identified to categorize subsets of immunosuppressive populations depleted. In addition, compounds targeting MDSC and Treg accumulation or function could also lead to systemic depletion of immune cells and generalized immunosuppression. In addition, for therapeutic success, it is critical that not only are MDSCs and Tregs depleted, but that Teff and CTLs are activated or released from T cell exhaustion. Also, as part of combination therapies with first and second line TB regimens, there is the likelihood compounds targeting MDSCs or Tregs or both will have unfavorable interactions with other drugs in the regimens. Therefore, further studies are required to achieve the goal of specific targeting of MDSCs and Tregs as HDT for TB.

Conclusions and Future Perspectives

Recent studies have provided new insights into the roles played by MDSCs and Tregs in the progression of TB. Although *M.tb* infection promotes antigen-specific T cell responses, robust immunosuppression provided by MDSCs and Tregs in the tuberculous granuloma may partly account for the persistence of *M.tb* and the limited efficacy of vaccines. They provide new therapeutic opportunities for shifting the immune system in favor of potent anti-mycobacterial responses. MDSCs serve multiple functions, from harboring *M.tb*, to promoting expansion of Tregs, to attenuating pro-mycobacterial host immune responses. In humans infected with *M.tb*, the adaptive immune response is delayed and bacteria specific T cells are only detectable 6 weeks after infection [26]. Tregs appear to play an important role in mediating this delay and facilitating establishment of infection. Tregs induced by viral, bacterial and parasitic pathogens may account for the reduced efficacy of BCG vaccine, particularly in settings endemic for helminths, malaria, HIV etc. Many aspects of immune suppression mediated by MDSCs and Tregs in TB still

remain unexplored. Deciphering of mechanisms and molecules used by suppressive networks that lead to pathogen persistence and disease progression is of paramount importance for the design of successful HDT and for boosting vaccine-induced protective immunity. Murine studies need to be translated to relevant aspects of human pathology. Establishing Tregs and MDSCs or their mediators as biomarkers for treatment monitoring also deserves further attention.

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