Emerging patterns of regulatory T cell function in tuberculosis

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Abstract

Tuberculosis (TB) is one of the top 10 causes of mortality worldwide from a single infectious agent and has significant implications for global health. In 2018, 1.5 million people died from TB worldwide and 440,000 of those were from India. The WHO End-TB strategy aims to reduce TB deaths by 95% and new TB cases by 90% by 2035, with a call for more basic research on TB pathogenesis and immunity. A major hurdle in the development of effective TB vaccines and therapies is the absence of defined immune-correlates of protection. In this context, the role of regulatory T cells (Treg), which are essential for maintaining immune homeostasis, is even less understood. This review aims to address this knowledge gap by providing an overview of the emerging patterns of Treg function in TB. The review also provides a comprehensive critical analysis of the key features of Treg cells in TB; highlights the importance of a balanced immune response as being important in TB and discusses the importance of probing not just Treg frequency but also qualitative aspects of Treg function as part of a comprehensive search for novel TB treatments.

Regulatory T cells: a brief introduction

Extensive experimental evidence shows that it is not only important to mount an effective immune response but equally crucial to efficiently control it. A vital cog in the immune regulation machinery is a class of $CD4^+$ T cells, termed as regulatory T cells or Treg cells. Treg cells have been extensively studied particularly in autoimmune disorders, as potential therapeutic targets. Tregs can be broadly classified as (a) thymic or tTregs/natural or nTregs, which originate in the thymus and, (b) induced or iTregs/peripheral or pTregs, which develop in the periphery during T cell activation. Development of nTregs is influenced by signal strength [1,2] co-stimulatory CD28 signaling, ICOS/ICOSL interactions, and thymic stromal lymphoprotein (TSLP) [3-5]. Transcription factor FoxP3 is a key regulator of Treg development, maintenance and suppressive function [6-10] and its expression in $CD4^+CD25^{hi}FoxP3^+$tTregs is positively regulated by IL-2 [11], transcription factors NFAT, STAT5 and Smad3 [12-14] and negatively regulated by PI3K, Akt and mTOR [15]. However, FoxP3 expression can also be induced upon exposure to non-self antigens in $CD4^+FoxP3^-$ conventional T cells, which then differentiate into $FoxP3^+$ Tregs known as pTregs/iTregs by a process regulated by cytokines TGF- β and IL-2 [16,17], suboptimal CD28 mediated co-stimulation [18], and sub-optimal TCR triggering [19, 20]. While nTregs maintain tolerance and homeostasis systemically, pTreg/iTregs are crucial for dampening over-exuberant antigen specific immune responses locally [21]. Inducible T regulatory type 1 or Tr1 cells, a sub-class of pTregs/iTregs, mediate suppressive effects via the immune-regulatory cytokine IL-10 [22]; cell surface markers CD49b and LAG3 promote Tr1 differentiation [23]; and IL-27, IL-6, IL-21, IL-10, immature DCs and plasmacytoid DCs promote Tr1 expansion [24-28]. nTreg suppression mechanisms can be contact-dependent or -independent (Figure 1). A breakdown in Treg suppression can occur due to (i) reduction in Treg frequencies, (ii) loss of Treg immunosuppressive capacity or due to (iii) resistance acquired by effector T cells (Teff) to Treg mediated suppression, with impact on a variety of clinical conditions (Table 1 and 2). This review focuses on how these mechanisms may contribute to disease in the context of tuberculosis (TB).

Tuberculosis

M. tuberculosis (Mtb) the causative agent of TB is well-adapted to the human host, such that it can lie dormant for several years (latent TB), sometimes a life time, without causing disease. Only 5-10% of subjects infected with Mtb progress to disease during their life. Activation of latent TB can be due to several reasons among which HIV co-infection is a major pre-disposing factor [68]. Although, Mtb is spread through aerosols and replicates in lung epithelial cells, it can also replicate in lymph nodes, bones, stomach, kidneys and other organs causing extra-pulmonary TB. In extreme cases *Mtb* can be systemically disseminated precipitating a potentially fatal condition known as miliary TB. Upon entering the host through aerosol, Mtb bacilli are taken up by alveolar macrophages by phagocytosis facilitated by cell surface receptors, e.g. toll like receptors (TLR), C-type lectin receptors (CLR), scavenger receptors (SR), complement receptors (CR) and Fc receptors [reviewed in 69] and replicate in macrophages in the lung parenchyma. Primed DCs traffic to the lymph node and trigger activation of adaptive immune cells, which are recruited to the lung and gradually an organized structure, the granuloma, begins to form, which comprises a caseous, necrotic core with replicating bacilli, surrounded by an inner ring of epithelioid interlocked macrophages, neutrophils and foam cells and an outer ring of T cells, B cells and NK cells [70]. The resolution of infection within the granuloma relies on host immune responses, which can potentially be impacted by Treg cell function. Indeed, the role of Tregs has been studied in the context of early acute stage of Mtb infection and the chronic phase of infection with evidence from mouse, primate and human studies, as summarized below.

Tregs in TB : Acute phase of infection: an analysis of animal model studies suggests early expansion to be detrimental. Mice models highlight the impact of Tregs on TB to be phase specific with Treg frequencies inadvertently high in the acute phase, which is detrimental for infection control [71-76]. Aerosol infection of mice with mycobacteria leads to activation of $CD4^+$ Teff cells by infected DCs in the pulmonary lymph node at approximately day 11 and subsequent expansion and accumulation of $CD4^+$ (effector and regulatory) T cells in the lungs by day 14-21 [71]. Significant disease associated lung pathology and cfu (colony forming units) burden can be observed at day 14-21 and this period can be classified as the early phase of infection in mice [71, 74, 75]. Time points subsequent to this, e.g. 4-7 wks post infection can be classified as late stages of infection [71, 74, 75]. Whilst timelines for early and late phases can vary with multiplicity of infection, in general 50-200 Mtb cfu results in increased Treg frequencies in lung and pulmonary lymph node at 10-21 days which is maintained till 60-127 days post infection [73,76]. This early expansion was found to be deleterious to emerging protective anti-TB Th responses [72-74, 76, 77]. Depletion of Tregs in C57BL/6 mice by systemic administration of anti-CD25 3 days prior to infection with BCG resulted in enhanced culture filtrate protein (CFP) specific $IFN\gamma^+$ and $IL\text{-}2^+CD4^+$ cells in lungs and spleen of BCG-infected mice 14 days post infection suggesting that presence of Treg cells hinders appearance of protective Th1 responses [74]. Also, adoptive transfer of $CD25^+$ Treg into Mtb infected mice leads to reduced frequencies of Mtb-specific Teff cells in the lungs at 14-17 days post aerosol infection [72]. Importantly, absence of protective Th1 responses due to expansion of Tregs leads to increased bacterial burden in the acute phase [73]. However, this dampening effect of Tregs on protective immune responses is transient and not evident in later stages of infection [71, 74, 75]. Depletion of $CD25^+$ Treg had no effect on cfu burden or lung pathology in BCG or Mtb Erdman infected mice at days 21 and 44 post infection [74]. Similar results were reported in another study, where Treg depletion in Mtb Erdman or Kurono infected DBA/2 mice reduced cfu at 2 weeks post infection but had no effect on bacterial burden or pathology subsequently, at 3 and 5 weeks [75]. It has now been demonstrated in mice that Mtb-specific Tregs are culled via IL-12 driven expression of T-bet by 32 days post infection; T-bet being known for its pro-apoptotic effects [71]. How Mtb infection drives this early expansion of Mtb-specific Tregs, which is beneficial to the pathogen remains to be elucidated.

Chronic phase: animal model studies show loss in Treg frequency or failure to recruit Tregs to site of infection can be detrimental. In contrast to the detrimental role of Tregs in the early / acute stage of infection in murine models**,** several studies in mouse and primate models highlight a potentially beneficial role for Tregs in the chronic phase of infection. Comparison of TB disease progression and pathology in TB resistant and TB susceptible mouse strains showed TB resistant mouse strains to have higher Treg frequencies and consequently less TB induced lung pathology in the chronic phase of the disease [78, 79] compared to TB sensitive mice, which recruit significantly fewer Tregs to the lung [79]. Interestingly infecting TB sensitive

C3HeN/FeJ mice with *M. maserensis* (environmental mycobacterium) resulted in a boost in Treg frequencies with a reduction in lung pathology and improved survival [78]. These observations have been corroborated in non-human primate models of TB infection, where cynomolgus macaques infected with 25 cfu of Mtb Erdman can either develop active TB or establish latency [80]. In this experimental system it was observed that macaques that developed latent TB had higher basal pre-infection Treg frequencies compared to animals that develop active disease [80]. In a separate study, IL-2 administered either pre- or post-Mtb infection in macaques resulted in Treg frequency expansion, which in turn led to reduced bacterial burden and TB induced pathology, suggesting that expansion of Treg cells in later stage of chronic TB infection can help control excessive TB induced inflammation [81].

Human studies: In contrast to animal model studies where changes in circulating Treg frequency can impact infection levels, reports of Treg frequencies in human TB are varied. Some studies show an increase in peripheral Treg frequencies in TB [55-58]. However, our study [61] and others [59, 60] found no differences in peripheral Treg frequencies between pulmonary TB patients and healthy controls. This disparity may be linked to differences in markers used for Treg delineation, which vary and can include, CD4 and CD25 [55, 56]; CD4, CD25 and FoxP3 [57, 59], a combination of CD4, CD45RA/CD45RO, CD127, CD25 and FoxP3 to identify memory Tregs [60, 61] or $CD4^+CD127^{lo}CD25^+FoxP3^+CD45RO^+Ki67^+$to identify activated Treg cells [60]. Beyond variation in markers used for definition, a further limitation of only tracking Treg frequency to define Treg function in a disease like TB, is the impact of trafficking; thus Treg frequencies have been shown to be higher at the site of infection in the broncheoalveolar lavage compared to that in the peripheral blood of pulmonary TB subjects [55, 82].

Chronic phase: novel qualitative studies of Treg function in humans highlight emergence of Treg resistant T effectors in chronic TB.

In contrast to the many studies that have probed Treg frequency, few human or animal model studies have analyzed qualitative aspects of Treg function in TB. Some studies have shown that Treg cells from pulmonary TB patients retain their capacity to suppress autologous Teff cells [83-85]. However, data from our laboratory shows that autologous suppression mediated by $CD4^+CD45RA^-CD25^+CD127^{lo}$memory Treg cells isolated from subjects with pulmonary TB in south India is significantly compromised [61]. By testing isolated Tregs from healthy controls on Teff isolated from TB subjects and vice versa, we demonstrated this impairment is not due to the loss of suppressive potential of Treg cells isolated from TB subjects; instead it is due to the effector cells from TB subjects acquiring resistance to Treg mediated suppression [61]. Thus, $CD127^{lo}CD25^+$ Treg cells from TB subjects were effective in suppressing Teff from healthy controls but not those from TB subjects; conversely, Treg isolated from healthy controls effectively suppressed autologous Teff but failed to suppress Teff from TB subjects [61]. Phenotypic analysis of the Treg resistant Teff isolated from TB subjects highlighted the presence of a significant proportion of highly activated cells that expressed HLA-DR and CD38; depletion of the $HLA\text{-}DR^+$ subset in particular, restored sensitivity of HLA-DR- Teff to autologous Treg suppression, thereby confirming that resistance of Teff from TB subjects to Treg mediated suppression was due to the presence of $HLA\text{-}DR^+$ cells [61]. The expansion of $HLA\text{-}DR^+CD4^+$ T cells in TB is driven by infection as anti-tubercular (anti-TB) treatment reduced the frequencies of $HLA\text{-}DR^+CD4^+$ T cells [61, 86, 87]; indeed we have shown that anti-TB treatment by dampening the frequency of $HLA\text{-}DR^+$ cells restores T effector cell sensitivity to autologous Treg cell mediated suppression [61]. Consequently, measuring $HLA\text{-}DR^+CD4^+$ T cell frequency can potentially be used to monitor treatment responses and predict efficacy of treatment [87]. In this context, our observation that $HLA\text{-}DR^+CD4^+$ T effectors isolated from subjects with pulmonary TB become resistant to Treg mediated suppression provides a mechanistic basis for how the expansion of $HLA\text{-}DR^+$ T effectors may be detrimental in TB (Figure 2, 61).

The observations of the emergence of Treg resistant Teff in TB is consistent with data from other chronic inflammatory conditions, particularly, autoimmune disorders (Table 2). $CD161^+$Th17 cells enriched in the synovial fluid of rheumatoid arthritis patients are resistant to Treg mediated suppression and their depletion restores suppression in *in vitro* cultures [65]. A similar phenomenon of the emergence of suppression resistant effectors has been reported in systemic lupus erythromatosis [88], multiple sclerosis [89], type-1 diabetes [63,

90] and juvenile idiopathic arthritis [91], with potentially varying mechanisms underpinning such resistance. In multiple sclerosis, it was attributed to high Teff cell derived granzyme B [89]; in type-1 diabetes due to downregulation of TGFβRII on Teff cells and consequently reduced TGFβ mediated suppression [90] and in juvenile idiopathic arthritis, like TB, due to expansion of activated $CD69^{+}HLA\text{-}DR^{+}$ Teff cells which were Treg suppression resistant [91].

Mechanisms that underpin Treg dysregulation in TB

(i) Evidence for exaggerated expression of cytokines that counterbalance Treg function

It is well recognized that proinflammatory cytokines can suppress the generation and function of Treg cells. By directly comparing the transcriptome of Treg resistant HLA-DR^{+} effector $CD4^{+}$ T cells isolated from TB subjects with that of the Treg sensitive fraction depleted of HLA-$DR^{+}CD4^{+}$ T cells, we provided first evidence that HLA-DR^{+} T effectors from TB express a number of pro-inflammatory cytokines including IL-2, IFNγ, CSF2, IL-17A and IL-22 (Figure 3). This exaggerated cytokine profile was noted in T effectors stimulated with both Mtb antigen as well as polyclonal stimulation [61]. Both IFNγ and IL-17A, although crucial for Mtb control [92, 93], are also recognized to counter-regulate Treg development and function and their exaggerated expansion therefore, could be one mechanism for Teff resistance to Treg control [94-96].

(II) ***Evidence for altered expression of cell surface molecules on Teff that are important for engaging with Treg cells***

Treg cells suppress via a variety of contact dependent and independent mechanisms (Figure 1). Some of the key molecules shown to be involved in promoting Treg suppression include CTLA-4, PD-1, CD39, CD73, PD-1, PD-L1, LAG3 etc. Several lines of data show some of these molecules, e.g. PD-1, PD-L1, CD39, CD73, LAG3 are altered in TB. PD-1 and PD-L1 expression is elevated in $CD4^{+}$ T cells from TB subjects compared to healthy controls [97]; CD39 and CD73 are increased in lung parenchymal $CD4^{+}$ T cells of Mtb infected mice [98] and LAG3 is increased in granuloma of macaques with active TB compared to those with latent TB [99]. However, the function of these molecules has not been specifically studied in the context of Treg suppression in TB. We provided some direct evidence for a role for PD-1/PD-L1 and β chemokine/CCR5 interactions. A comparative RNA seq analysis of Treg suppression sensitive (HLA-DR^{-} Teff) and suppression resistant (comprising HLA-DR^{+} Teff $^{+}$ and HLA-DR^{-} Teff) cells isolated from subjects with pulmonary TB showed elevated expression of PD-L1 and β-chemokines in the suppression sensitive HLA-DR^{-} Teff fraction (Figure 2). Also, blockade of PD-1/PD-L1 and β-chemokine/CCR5 interactions resulted in loss of Treg suppression in these cells pointing to the importance of these pathways in maintaining Treg mediated homeostasis in TB (Figure 2). PD-1/PD-L1 interactions and β-chemokine/CCR5 interactions have been previously implicated to play a role in Treg mediated suppression [100, 101]. CCL3 and CCL4 secreted by Tregs serve as chemoattractants for Teff cells. $CD4^{+}$ Teff cells from mice deficient in CCL3 and CCL4 fail to migrate and come in contact with Treg cells [101]. Moreover, Tregs from type-1 diabetes patients are deficient in CCL3 and CCL4 and this compromises their ability to suppress [101].

Apart from differences in PD-L1 and β-chemokine levels (Figure 2), our transcriptome analysis of Treg sensitive HLA-DR^{-} and suppression resistant HLA-DR^{+} cells identified several additional cell surface markers (CD46, TRAIL, TRAF1, TRAF3, FASLG, CD30, SEMA7A) (Figure 3), some of which have previously been implicated in Treg function [102-105]. Engagement of complement receptor CD46 results in suppression of bystander $CD4^{+}$ T cells via an IL-10 dependent mechanism [102, 103]. CD46 cross-linking also suppresses mycobacteria specific $CD4^{+}$ T cell responses [106]. TRAIL is a regulator of T cell activation; its absence leads to autoimmunity and reduction in Treg frequencies while its presence dampens Th1 responses and boosts Tregs [104]. TRAF1 inhibits Th2 differentiation [107]; TRAF3 controls proximal T cell activation events and its absence in mice leads to elevated thymus derived Treg cell frequencies [105]; FASLG is a marker for T cell activation and is expressed on Th1 cells [108, 109]; Sema7a and CD30 have been implicated in Th1 and Th17 differentiation [110, 111]. However, a role for these pathways impacting T effector function in TB remains to be elucidated.

(III) Signal strength

A third potentially important consideration in the mechanisms that underpin how activated, HLA-DR$^+$ T effectors become resistant to Treg cells may be linked to the quality and strength of the primary signal that activates effector cells. The strength of activating signal shapes the nature of the immune response, with high signal strength leading to Th1 and low signal strength to Th2, Tfh and memory T cell differentiation [reviewed in 112, 113]. Previous studies show that effectors activated by a very strong signal strength become refractory to suppression mediated by Treg cells- co-culture of human CD25$^-$ Teff and autologous CD25$^+$ Treg resulted in suppression only when stimulated with soluble anti-CD3 (weak TCR signal) and not when activated with plate-bound anti-CD3 (strong TCR signal) [114]. Whether this is pertinent in the context of TB remains to be tested. What has been demonstrated from mouse studies is that persistently activated CD4 T cells specific for the secretory Mtb antigen ESAT6, which is expressed in abundance throughout infection, fail to confer protection, whereas, CD4$^+$ T cells specific for an Mtb antigen that has more controlled expression, e.g. Ag85B, can confer protection greater protection [115]. The failure of ESAT6 specific cells to confer protection was linked to the fact that these cells are more exhausted and terminally differentiated; i.e. express higher KLRG1, lower CCR7, CD127 and CD62L; compared to Ag85B specific cells [115]. Whether Treg resistant HLA-DR$^+$ Teff cells isolated from TB subjects arise due to persistent antigen stimulation and bear markers of exhaustion remains to be confirmed.

Summary and future directions

It appears now from studies in animal models and humans that in TB the role of Tregs, both nTreg and antigen specific, has several dimensions. While Tregs might delay the appearance of protective Th responses especially during the early stages of infection; their function in the chronic stage of TB disease is consistent with their known primary function that is linked to control of exaggerated inflammation, which if unchecked, can contribute to disease pathology [116, 117]. The immune response in TB is clearly disordered and the theme of balance between protective and pathogenic responses has been visited in the past [118]. In fact, a balance between Th1/Th17 and immune-regulatory responses is associated with better clearance of Mtb infection [119]. In this context, the expansion of HLA-DR$^+$Teff cells in TB is a likely marker for inflammation associated with enhanced disease risk [120]. It has now been demonstrated that this expanded subset exhibits resistance to suppression mediated by natural Treg cells [61]. The putative role of pro-inflammatory cytokines (IFNγ, IL-17A, IL-2, CSF2 and IL-22), β-chemokines and PD-1/PD-L1 interactions in modulating T effector resistance to Treg suppression in TB has been identified (Figure 2). This calls for further analysis of the mechanisms that are important in maintaining balance between inflammation and immune-regulation in TB.

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Figure Legends

Figure 1: Mechanisms of Treg mediated suppression. Primary Treg suppression mechanisms include (1) acting as sink for IL-2 due to constitutive high expression of IL-2R and consequently depriving effector T cells of the crucial cytokine [121]; (2) secretion of immune-suppressive cytokines IL-10, TGFβ and IL-35 [122,123]; (3) Granzyme-B dependent killing of target cells [124]; (4) inhibitory signalling through binding of CTLA-4 on Tregs and CD80/86 on DCs and reverse signalling via this interaction leading to elevated levels of IDO in DCs which eventually deplete tryptophan and starve effector T cells [125, 126]; (5) binding of LAG3 to MHC-II molecules on DCs causing reduction in Ag presentation [127]; (6) suppression due to interaction of PD-1 on Tregs and PD-L1 on target cells [61, 100]; (7) extracellular adenosine generated from ATP in concert by cell surface CD39 and CD73 (ecto-5'-nucleotidase) interacts with A2AR on effector T cells and suppresses their function by increasing cAMP levels [128, 129]; (8) chemokines CCL3 and CCL4 secreted by Tregs bind to CCR5 on effector cells triggering their migration and subsequent suppression [61,

101].

Figure 2: A diagrammatic model which highlights the difference in Treg suppression in healthy and latently infected individuals and active TB subjects in context of expansion of HLA-DR$^+$CD4$^+$memory T cells. Individuals infected with TB can either clear the bacteria, become latently infected or come down with active TB disease. There is also a possibility of reactivation of TB in latently infected subjects. The reasons for this can be HIV co-infection, treatment with check-point inhibitors like anti-PD-1, therapies such as anti-TNF for rheumatoid arthritis etc. HLA-DR$^+$ activated cells are low in healthy and latently infected individuals and Treg suppression is good. However, in active TB, HLA-DR$^+$CD4$^+$ T cells expand and Treg mediated suppression becomes poor. The Treg suppression pathways that are rendered inactive in TB are the PD-1/PD-L1 and β-chemokine-CCR5 dependent. The reason for their becoming inactive could be possible counter-regulation by IL-2, IL-17A, IFNγ, IL-22 that are secreted by the expanded HLA-DR$^+$CD4$^+$ T cells.

Figure 3: Treg suppression resistant total Teff cells and Treg suppression sensitive HLA-DR- Teff cells have distinct expression patterns with respect to certain cytokines and cell surface receptors. Total Teff and HLA-DR- Teff were sorted by flow cytometry from 5 pulmonary TB patients. Cells were activated for different times with anti-CD3/CD28 mitogenic beads, RNA was isolated and an RNA-Seq was performed. Expression at each time was compared to baseline unactivated control to arrive at a DEG list. A final DEG list was prepared applying a cutoff of log2 fold change +/-2.5 and $p < 0.05$ (for details on procedure and complete DEG list please see Ahmed A et al., 2018). The DEG list was further mined to study expression of cytokines, chemokines, activation markers, cell surface markers and transcription factors. A summary of these results is shown. The numbers in boxes denote log2 fold change for expression at 2, 24 and 96 hrs compared to expression at baseline. For details of the complete DEG list, kindly see [61]

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Table 1: A summary of clinical conditions both autoimmune (peach highlighted) and infection (blue highlighted) where Treg frequency and function are compromised.

Sl. No.	Clinical Condition	Finding	Reference
1.	Rheumatoid Arthritis (RA)	Polymorphisms in FOXP3 gene associated with reduced frequency of Treg, TGFβ and IL-10, in RA	*[29]*
		Increased circulating HLA-DR^+Tregs or inflammation-associated Tregs which are suppressive but have similar TCR repertoire as pathogenic $CD4^+$ T cells	*[30]*
		Reduced frequencies of nTreg in patients with RA	*[31]*
		Tregs unable to suppress spontaneous generation of TNFα in synovial cells of RA patients due to reduced expression of CTLA-4 and LFA-1	*[32]*

Sl. No.	Clinical Condition	Finding	Reference
2.	Multiple Sclerosis (MS)	$CD4^+CD25^+$ Treg cells/Treg-derived exosomes from multiple sclerosis patients are inefficiently suppressive. Circulating exosomes with significantly high miRNA let-7i in MS patients, inhibit Treg function through an IGFR1 and TGFBR1 mechanism.	*[33-35]*
		$CD25^+CD127^{low}$ Treg development and function are perturbed. $CD39^+FoxP3^+$ memory Treg are diminished in MS patients. Expression of PD-1 is high on theses Tregs in MS, suggesting possible exhaustion and compromised function.	*[36,37]*
3.	Systemic Lupus Erythromatosis (SLE)	$CD25^+Lag3^+$ T cells, expressing FoxP3 and IL-17A, but not being suppressive are increased in patients with SLE. The frequency of $CD25^+Lag3^+$ cells positively correlates with SLE disease activity.	*[38]*
4.	Type 1 Diabetes	Reduced suppressive function of Treg cells in Type 1 diabetes patients possibly due to reduced CD39 expression on memory Treg cells.	*[39]*
		Differentiation and stability of Tregs is impaired in Type 1 diabetes through a miRNA-1423p dependent mechanism.	*[40]*

Sl. No.	Clinical Condition	Finding	Reference
		FoxP3 expression declines with type 1 diabetes disease progression suggesting loss in Treg function. The rate of loss is greatest in Peptidase inhibitor -16 or $Pi16^+$ Treg cells	*[41]*
5.	Malaria	$FoxP3^+$Treg cells increase in humans and mice during blood stage malaria and hamper Th and Tfh-B cell interactions.	*[42]*
		Frequency of $FoxP3^+$ Tregs declines in children with age in high exposure malaria settings.	*[43]*
6.	Dengue	Treg frequencies are higher in mild cases of dengue compared to moderate cases and healthy controls.	*[44]*
		Treg frequencies in acute dengue fever are high and most of the expanded Treg population comprises of naïve Tregs with poor suppressive potential.	*[45]*
7.	HIV	HIV infected paediatric slow progressors have higher Treg absolute numbers with a suppressive phenotype compared to rapid progressors.	*[46]*
		Depletion of $CD4^+CD25^{hi}CD62L^{hi}$ Tregs are depleted in HIV infection and this correlates with immune activation.	*[47]*

Sl. No.	Clinical Condition	Finding	Reference
		HIV+ elite suppressors maintain higher levels of Treg and lower immune activation compared to progressors.	*[48]*
		Frequency of PD-1^{+} Tregs increases in HIV and blockade of the PD-1/PD-L1 pathway increases TGF-β and IL-10 in CD4^{+}CD25$^{\text{hi}}$CD127$^{\text{lo}}$Treg cells.	*[49]*
		Individuals who do not respond to ART have fewer dysfunctional Tregs with defects in mitochondrial function compared to healthy controls and HIV patients who respond to ART.	*[50]*
8.	Candida infection	*Candida albicans* infection in a mouse model drives expansion of Tregs which corresponds with increased fungal burden. Expanded Tregs suppress Th1 and Th2 but promote pathogenic Th17 responses.	*[51, 52]*
9.	Leishmaniasis	Foxp3^{+}IL-10^{+} Treg cells are enriched in bone marrow of visceral leishmaniasis patients with high parasite load compared to those with low parasite load. Frequency of CD4^{+}CD25$^{\text{hi}}$FoxP3^{+} Treg cells correlates with parasite load in Kala-azar patients infected with *Leishmania donovani*.	*[53, 54]*

Sl. No.	Clinical Condition	Finding	Reference
10.	Tuberculosis	While some studies have found Treg frequencies to increase in blood and lungs of TB patients, others have found them to remain unchanged.	*[55-61]*

Table 2: A summary of clinical conditions where Teff susceptibility to Treg mediated suppression is altered.

Sl. No	Clinical Condition	Finding	Reference
1.	Type I Diabetes	Resistance of Teff cells to Treg mediated suppression via faster activation of STAT3 signaling post TCR stimulation in Type-1 diabetes patients	*[62]*
		Effector T cells from type 1 diabetes patients are resistant to suppression mediated by $CD4^+CD25^+$ Treg cells.	*[63, 64]*
2.	Rheumatoid Arthritis	Synovial $CD161^+$Th17 cells are resistant to Treg mediated suppression in rheumatoid arthritis patients	*[65]*
3.	HIV Infection	Increased sensitivity of $CD4^+CD25^-$ Teff cells to Treg mediated suppression in HIV^+ asymptomatic individuals compared to progressors. HLA-B*27 and HLA-B*57 restricted $CD8^+$ T cells associated with protection against HIV are not suppressed by Treg cells.	*[66, 67]*

Sl. No	Clinical Condition	Finding	Reference
4.	Tuberculosis	HLA-DR^{+}CD4^{+} memory T cells which are IFN-γ^{hi}IL-2^{hi}IL-17^{hi}IL-22^{hi} are resistant to Treg mediated suppression in TB patients.	*[61]*

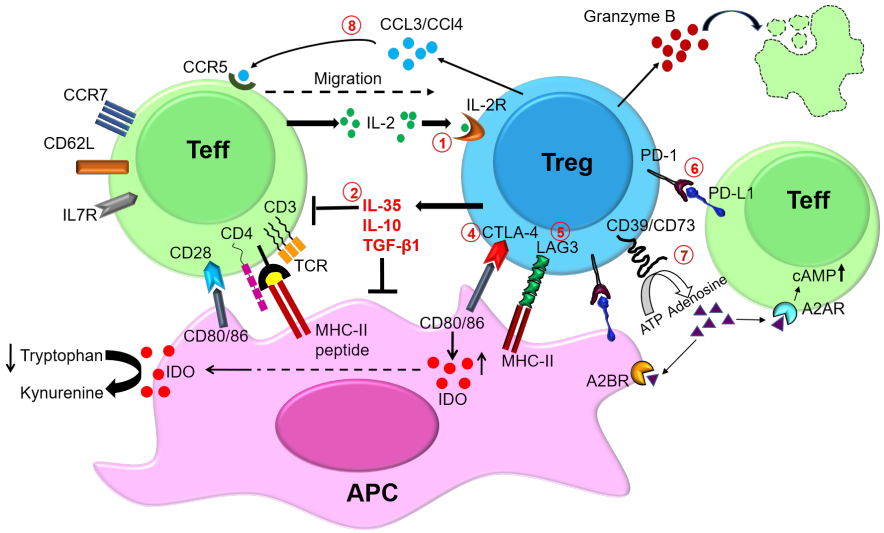

Figure 1: Mechanisms of Treg mediated suppression. Primary Treg suppression mechanisms include (1) acting as sink for IL-2 due to constitutive high expression of IL-2R and consequently depriving effector T cells of the crucial cytokine [121]; (2) secretion of immune-suppressive cytokines IL-10, TGFβ and IL-35 [122,123]; (3) Granzyme-B dependent killing of target cells [124]; (4) inhibitory signalling through binding of CTLA-4 on Tregs and CD80/86 on DCs and reverse signalling via this interaction leading to elevated levels of IDO in DCs which eventually deplete tryptophan and starve effector T cells [125, 126]; (5) binding of LAG3 to MHC-II molecules on DCs causing reduction in Ag presentation [127]; (6) suppression due to interaction of PD-1 on Tregs and PD-L1 on target cells [61, 100]; (7) extracellular adenosine generated from ATP in concert by cell surface CD39 and CD73 (ecto-5'-nucleotidase) interacts with A2AR on effector T cells and suppresses their function by increasing cAMP levels [128, 129]; (8) chemokines CCL3 and CCL4 secreted by Tregs bind to CCR5 on effector cells triggering their migration and subsequent suppression [61, 101].

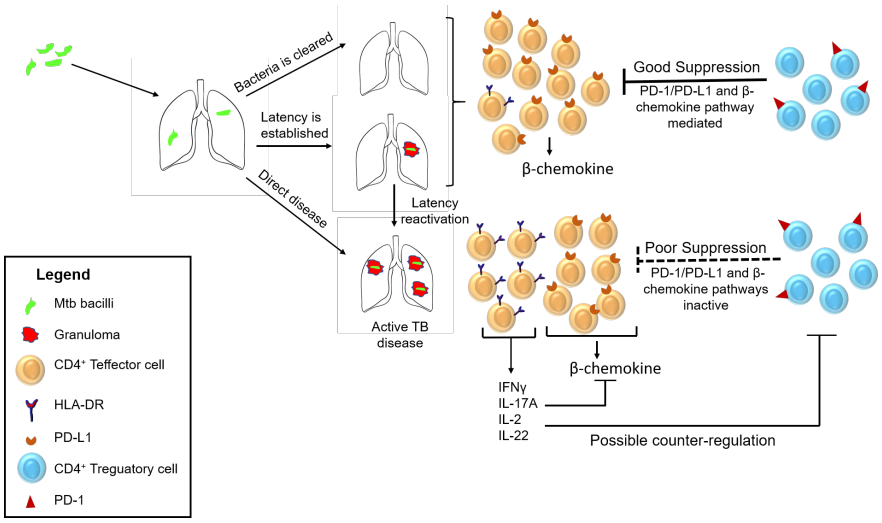

Figure 2: A diagrammatic model which highlights the difference in Treg suppression in healthy and latently infected individuals and active TB subjects in context of expansion of HLA-DR$^+$CD4$^+$ memory T cells. Individuals infected with TB can either clear the bacteria, become latently infected or come down with active TB disease. There is also a possibility of reactivation of TB in latently infected subjects. The reasons for this can be HIV co-infection, treatment with check-point inhibitors like anti-PD-1, therapies such as anti-TNF for rheumatoid arthritis etc. HLA-DR$^+$ activated cells are low in healthy and latently infected individuals and Treg suppression is good. However, in active TB, HLA-DR$^+$CD4$^+$ T cells expand and Treg mediated suppression becomes poor. The Treg suppression pathways that are rendered inactive in TB are the PD-1/PD-L1 and β-chemokine-CCR5 dependent. The reason for their becoming inactive could be possible counter-regulation by IL-2, IL-17A, IFNγ, IL-22 that are secreted by the expanded HLA-DR$^+$CD4$^+$ T cells.

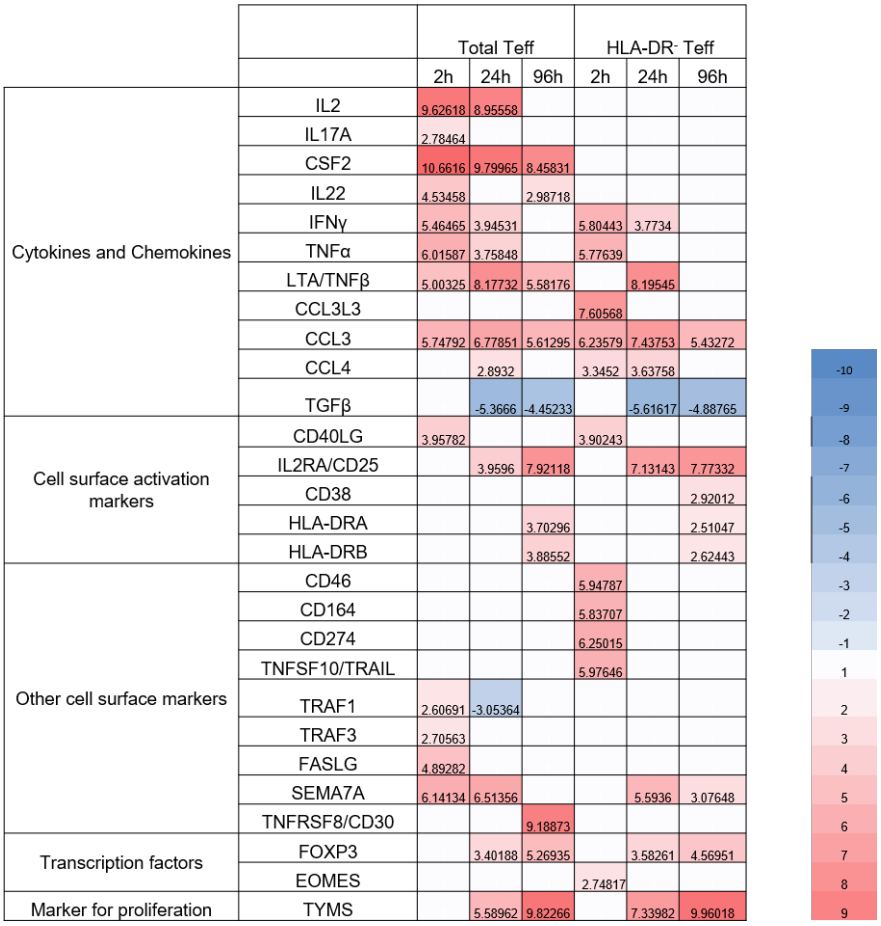

Figure 3: Treg suppression resistant total Teff cells and Treg suppression sensitive HLA-DR- Teff cells have distinct expression patterns with respect to certain cytokines and cell surface receptors. Total Teff and HLA-DR- Teff were sorted by flow cytometry from 5 pulmonary TB patients. Cells were activated for different times with anti-CD3/CD28 mitogenic beads, RNA was isolated and an RNA-Seq was performed. Expression at each time was compared to baseline unactivated control to arrive at a DEG list. A final DEG list was prepared applying a cutoff of log2 fold change +/-2.5 and $p < 0.05$ (for details on procedure and complete DEG list please see Ahmed A et al., 2018). The DEG list was further mined to study expression of cytokines, chemokines, activation markers, cell surface markers and transcription factors. A summary of these results is shown. The numbers in boxes denote log2 fold change for expression at 2, 24 and 96 hrs compared to expression at baseline. For details of the complete DEG list, kindly see [61]