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Emerging patterns of regulatory T cell function in tuberculosis

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Summary

Tuberculosis (TB) is one of the top 10 causes of mortality worldwide from a single infectious agent and has significant implications for global health. 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 (T_{reg}), 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 T_{reg} function in TB. Increasing evidence from studies, both in animal models of infection and TB patients, points to the fact the role of T_{regs} in TB is dependent on disease stage. While T_{regs} might expand and delay the appearance of protective responses in the early stages of infection, their role in the chronic phase perhaps is to counter-regulate excessive inflammation. New data highlight that this important homeostatic role of T_{regs} in the chronic phase of TB may be compromised by the expansion of activated human leucocyte antigen D-related (HLA-DR)$^+$CD4$^+$ suppression-resistant effector T cells. This review provides a comprehensive and critical analysis of the key features of T_{reg} cells in TB; highlights the importance of a balanced immune response as being important in TB and discusses the importance of probing not just T_{reg} frequency but also qualitative aspects of T_{reg} function as part of a comprehensive search for novel TB treatments.

Keywords: HLA-DR, T_{eff}, T_{reg}, tuberculosis

Regulatory T (T_{reg}) 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 regulatory T (T_{reg}) cells. T_{reg} cells have been extensively studied, particularly in autoimmune disorders, as potential therapeutic targets. T_{regs} can be broadly classified as (a) thymic or tT_{regs}/natural or nT_{regs}, which originate in the thymus, and (b) induced or iT_{regs}/peripheral or pT_{regs}, which develop in the periphery during T cell activation. Development of nT_{regs} is influenced by signal strength [1,2] co-stimulatory CD28 signalling, inducible T cell co-stimulator (ICOS)/ICOS ligand (ICOS/ICOSL) interactions and thymic stromal lymphoprotein (TSLP) [3–5]. Transcription factor forkhead box protein 3 (FoxP3) is a key regulator of T_{reg} development, maintenance and suppressive function [6–10] and its expression in CD4$^+$CD25^{high}FoxP3$^+$ tT_{regs} is positively regulated by IL-2 [11], transcription factors nuclear factor of activated T cells (NFAT), signal transducer and activator of transcription 5 (STAT-5) and 'small' and 'mothers against decapentaplegic' homologue 3 (SMAD3) [12–14] and negatively regulated by phosphatidyl inositol-3 kinase (PI3K), protein kinase B (Akt) and mammalian target of rapamycin (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$^+$ T_{regs} known as pT_{regs}/iT_{regs} by a process regulated by cytokines

transforming growth factor (TGF)-β and interleukin (IL)-2 [16,17], suboptimal CD28-mediated co-stimulation [18] and suboptimal T cell receptor (TCR) triggering [19,20]. While nT_{regs} maintain tolerance and homeostasis systemically, pT_{regs}/iT_{regs} are crucial for dampening over-exuberant antigen-specific immune responses locally [21]. Inducible T regulatory type 1 (Tr1) cells, a subclass of pT_{regs}/iT_{regs}, mediate suppressive effects via the immune-regulatory cytokine IL-10 [22]; cell surface markers CD49b and lymphocyte-activation gene 3 (LAG3) promote Tr1 differentiation [23]; and IL-27, IL-6, IL-21, IL-10, immature dendritic cells (DCs) and plasmacytoid DCs promote Tr1 expansion [24–28]. Other iT_{reg} subsets include T cell immunoreceptor with immunoglobulin (Ig) and immunoreceptor tyrosine-based inhibition motif (ITIM) domain or TIGIT-1^+ cells, which express high levels of T_{reg} signature genes [e.g. IL-10, cytotoxic T lymphocyte antigen 4 (CTLA-4), LAG3, FoxP3, etc.] capable of suppressing Th1 and Th17 responses via a mechanism involving expression of fibrinogen-like protein 2 (Fgl2) post-ligation of TIGIT with CD155 [29]; IL-35-producing suppressive T (Tr35) cells that suppress via secretion of regulatory cytokine IL-35 [30]; and IL-10-producing non-pathogenic Th17 cells that are capable of controlling autoimmune inflammation, e.g. rheumatoid arthritis, inflammatory bowel disease, etc. [31].

T_{reg} suppression mechanisms can be contact-dependent or -independent (Fig. 1). A breakdown in T_{reg} suppression can occur due to (i) reduction in T_{reg} frequencies, (ii) loss of T_{reg} immunosuppressive capacity or due to (iii) resistance acquired by effector T cells (T_{eff}) to T_{reg}-mediated suppression, with impact on a variety of clinical conditions (Tables 1 and 2) in addition to TB (Table 3). This review focuses on how these mechanisms may contribute to disease in the context of TB.

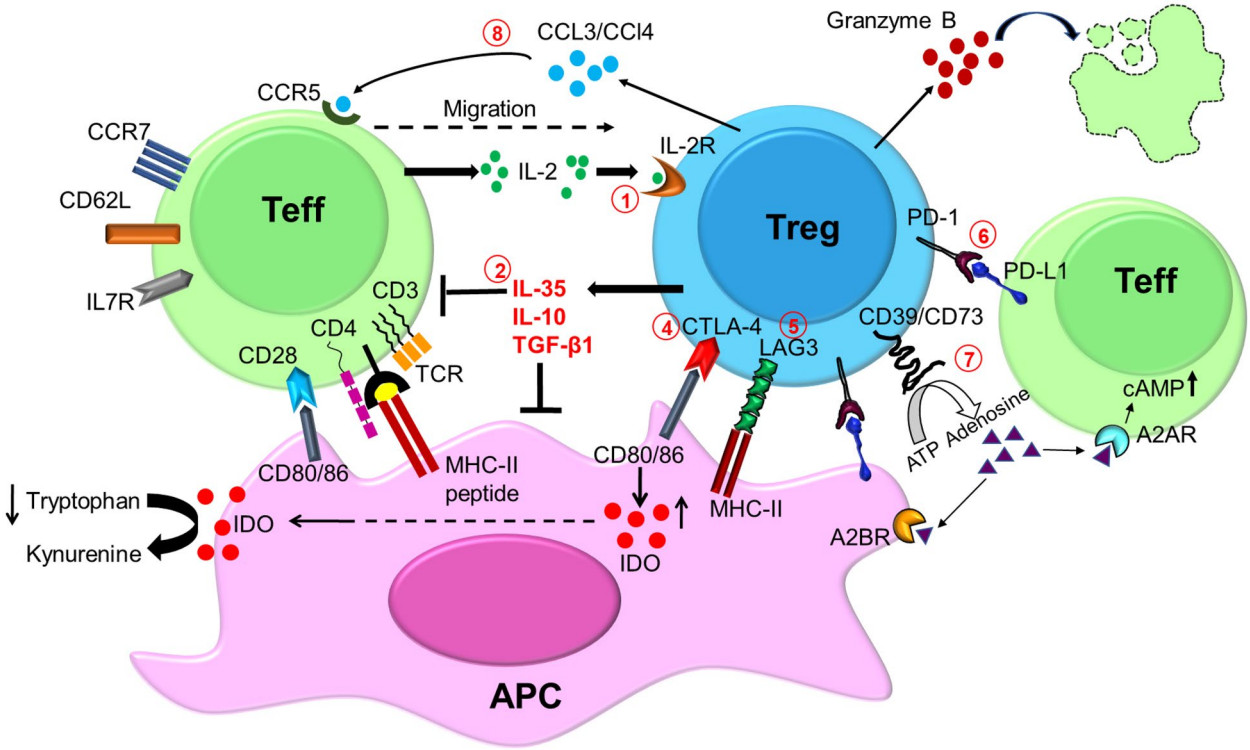


Fig. 1. Mechanisms of T_{reg}-cell mediated suppression. Well recognised and studied T_{reg} suppression mechanisms include (1) acting as a sink for interleukin (IL)-2 due to constitutive high expression of IL-2R and consequently depriving effector T cells of the crucial cytokine [125]; (2) secretion of immune-suppressive cytokines IL-10, transforming growth factor (TGF)-β and IL-35 [126,127]; (3) granzyme-B dependent killing of target cells [128]; (4) inhibitory signalling through binding of cytotoxic T lymphocyte antigen 4 (CTLA-4) on T_{regs} and CD80/86 on dendritic cells (DCs) and reverse signalling via this interaction leading to elevated levels of indoleamine 2,3-dioxygenase (IDO) in DCs which eventually depletes tryptophan and starves effector T cells [129,130]; (5) binding of lymphocyte-activation gene 3 (LAG3) to major histocompatibility complex (MHC)-II molecules on DCs causing reduction in antigen presentation [131]; (6) suppression due to interaction of programmed cell death 1 (PD-1) on T_{regs} and programmed cell death ligand 1 (PD-L1) on target cells [64,99]; (7) extracellular adenosine generated from adenosine triphosphate (ATP) in concert by cell surface CD39 and CD73 (ecto-5'-nucleotidase) interacts with adenosine A_{2A} receptor (A2AR) on effector T cells and suppresses their function by increasing cAMP levels [132,133]; (8) chemokine (C-C motif) ligand 3 (CCL3) and CCL4 secreted by T_{regs} bind to C-C chemokine receptor type 5 (CCR5) on effector cells triggering their migration and subsequent suppression [64,100].

Table 1. A summary of clinical conditions both autoimmune (peach highlighted) and infection (blue highlighted) where T_{reg} frequency and function are compromised.

Sl. No.	Clinical Condition	Finding	Reference
1.	Rheumatoid arthritis (RA)	Polymorphisms in FoxP3 gene associated with reduced frequency of T_{reg}, TGF-β and IL-10 in RA.	[32]
		Increased circulating HLA-DR+ T_{regs} or inflammation-associated T_{regs} which are suppressive but have similar TCR repertoire as pathogenic CD4+ T cells.	[33]
		Reduced frequencies of nT_{reg} in patients with RA.	[34]
		T_{regs} unable to suppress spontaneous generation of TNF-α in synovial cells of RA patients due to reduced expression of CTLA-4 and LFA-1.	[35]
2.	Multiple Sclerosis (MS)	CD4+CD25+ T_{reg} cells/T_{reg}-derived exosomes from MS patients are inefficiently suppressive. Circulating exosomes with significantly high miRNA let-7i in MS patients, inhibit T_{reg} function through an IGFR1 and TGFBR1 mechanism.	[36-38]
		$CD25+CD127^{low}$ T_{reg} development and function are perturbed. CD39+FoxP3+ memory T_{reg} are diminished in MS patients. Expression of PD-1 is high in these T_{regs} in MS, suggesting possible exhaustion and compromised function.	[39,40]
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.	[41]
4.	Type 1 Diabetes	Reduced suppressive function of T_{reg} cells in type 1 diabetes patients possibly due to reduced CD39 expression on memory T_{reg} cells.	[42]
		Differentiation and stability of Tregs is impaired in Type 1 diabetes through a miRNA-1423p-dependent mechanism.	[43]
		FoxP3 expression declines with type 1 diabetes disease progression, suggesting loss in T_{reg} function. The rate of loss is greatest in Peptidase inhibitor (Pi)-16 or Pi16+ T_{reg} cells.	[44]
5.	Malaria	FoxP3+T_{reg} cells increase in humans and mice during blood stage malaria and hamper Th and Tfh–B cell interactions.	[45]
		Frequency of FoxP3+ T_{regs} declines in children with age in high exposure malaria settings.	[46]
6.	Dengue	T_{reg} frequencies are higher in mild cases of dengue compared to moderate cases and healthy controls.	[47]
		T_{reg} frequencies in acute dengue fever are high and most of the expanded T_{reg} population is comprised of naive T_{regs} with poor suppressive potential.	[48]
7.	HIV	HIV-infected paediatric slow progressors have higher T_{reg} absolute numbers with a suppressive phenotype compared to rapid progressors.	[49]
		$CD4+CD25^{high}CD62L^{high}$ T_{regs} are depleted in HIV infection and this correlates with immune activation.	[50]
		HIV+ elite suppressors maintain higher levels of T_{reg} and lower immune activation compared to progressors.	[51]
		Frequency of PD-1+ T_{regs} increases in HIV and blockade of the PD-1/PD-L1 pathway increases TGF-β and IL-10 in $CD4+CD25^{high}CD127^{low}T_{reg}$ cells.	[52]
		Individuals who do not respond to ART have fewer and dysfunctional T_{regs} with defects in mitochondrial function compared to healthy controls and HIV patients who respond to ART.	[53]
8.	Candida infection	*Candida albicans* infection in a mouse model drives expansion of T_{regs} which corresponds with increased fungal burden. Expanded T_{regs} suppress Th1 and Th2 but promote pathogenic Th17 responses	[54,55]
9.	Leishmaniasis	Foxp3+IL-10+ T_{reg} cells are enriched in bone marrow of visceral leishmaniasis patients with high parasite load compared to those with low parasite load.	[56,57]
		Frequency of $CD4+CD25^{high}$FoxP3+ T_{reg} cells correlates with parasite load in Kala-azar patients infected with *Leishmania donovani*.	

T_{reg} = regulatory T cell; ART = antiretroviral therapy; FoxP3 = forkhead box protein 3; TGF = transforming growth factor; IL-10 = interleukin 1; HLA-DR = human leucocyte antigen D-related; CTLA-4 = cytotoxic T lymphocyte antigen 4; LFA-1 = lymphocyte function-associated antigen 1; IGFR1 =insulin like growth factor 1 receptor; LAG3 =lymphocyte activation gene 3; Th = T helper; Tfh = T follicular helper cell; PD-1 = programmed cell death protein 1; PD-L1 = programmed cell death ligand 1.

Tuberculosis

Mycobacterium tuberculosis (Mtb), the causative agent of TB, can either cause latent or active TB disease, with the majority of people developing asymptomatic infection, commonly referred to as latent TB. However, increasing evidence shows that the asymptomatic state of TB is not necessarily a condition where TB bacilli are dormant, and therefore

Table 2. A summary of clinical conditions where T_{eff} susceptibility to T_{reg}-mediated suppression is altered.

Sl. no.	Clinical condition	Finding	Reference
1.	Type I diabetes	Resistance of T_{eff} cells to T_{reg}-mediated suppression via faster activation of STAT-3 signalling post-TCR stimulation in type 1 diabetes patients.	[58]
		Teffs from type 1 diabetes patients are resistant to suppression mediated by CD4+CD25+ T_{reg} cells.	[59,60]
2.	Rheumatoid arthritis (RA)	Synovial CD161+Th17 cells are resistant to T_{reg}-mediated suppression in RA patients.	[61]
3.	HIV infection	Increased sensitivity of CD4+CD25– T_{eff} cells to T_{reg}-mediated suppression in HIV+ asymptomatic individuals compared to progressors.	[62,63]
		HLA-B*27 and HLA-B*57 restricted CD8+ T cells associated with protection against HIV are not suppressed by T_{reg} cells.	
4.	Tuberculosis	HLA-DR+CD4+ memory T cells which are IFN-γ^{high}IL-2^{high}IL-17^{high}IL-22^{high} are resistant to T_{reg} -mediated suppression in TB patients.	[64]

T_{reg} = regulatory T cell; T_{eff} = effector T cells; TB = tuberculosis; IFN = interferon; IL = interleukin; HLA-DR = human leucocyte antigen D-related; STAT-3 = signal transducer and activator of transcription-3; TCR = T cell receptor.

the term 'latent TB' may be misleading. Indeed, radiography and positron emission tomography/computed tomography (PET/CT) scans of subjects with asymptomatic Mtb infection highlight a condition that is highly heterogeneous, where some subjects have lung lesions from which viable, metabolically active bacteria can be isolated [81], while others lack detectable lung lesions. Irrespective of this heterogeneity, only 5–10% of subjects with latent asymptomatic TB are known to progress to active TB disease during their lifetime, with the mechanisms driving such progression being an area of active research that, in turn, is limited by the lack of definitive correlates of protection, which is beyond the scope of this review. Progression from latent to active TB can be due to several reasons, among which HIV co-infection is a major predisposing factor [82]. 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 extrapulmonary 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 [83]) 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 core with replicating bacilli, surrounded by an inner ring of epithelioid interlocked macrophages, neutrophils and foam cells and an outer ring of T, B and NK cells [84]. With time, necrosis takes place and accumulation of necrotic material leads to formation of a caseum and the granuloma is known as a 'caseating granuloma', which can also undergo cavitation leading to Mtb dissemination [84,85]. The resolution of infection within the granuloma relies upon host immune responses, which can potentially be impacted by T_{reg} cell function. Indeed, the role of T_{regs} has been studied in the context of the early acute stage of Mtb infection and the chronic phase of infection with evidence from mouse, primate and human studies, as summarized below.

T_{regs} in TB

Acute phase of infection: an analysis of animal model studies suggests early expansion to be detrimental. Mouse models highlight the impact of T_{regs} on TB to be phase-specific with T_{reg} frequencies inadvertently high in the acute phase, which is detrimental for infection control [65–70]. Aerosol infection of mice with mycobacteria leads to activation of $CD4^+$ T_{eff} 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 days 14–21 [65]. Significant disease-associated lung pathology and colony-forming units (CFU) burden can be observed at days 14–21, and this period can be classified as the early phase of infection in mice [65,69,70]. Time-points subsequent to this, e.g. 4–7 weeks post-infection, can be classified as late stages of infection [65,69,70]. While time-lines for early and late phases can vary with multiplicity of infection, in general 50–200 Mtb CFU results in increased T_{reg} frequencies in lung and pulmonary lymph node at 10–21 days, which is maintained until 60–127 days post-infection [67,68]. This early expansion was found to be deleterious to emerging protective anti-TB Th responses [66–69]. Depletion of T_{regs} in C57BL/6 mice by systemic administration of anti-CD25 three days prior to infection with Bacille Calmette–Guérin (BCG) resulted in enhanced culture filtrate protein (CFP)-specific IFN-γ^+ and IL-2^+ $CD4^+$ cells in lungs and spleen of BCG-infected mice

Table 3. A summary of findings on T_{reg} frequency and function from animal models of infection and TB patients

		Findings		
	Study model	T_{reg} frequency	T_{reg} function	Refs
Mice	Mice infected with Mtb H37Rv via aerosol.	ESAT-6-specific FoxP3+ STAT-3 expand at day 21 and contract by days 32–35 post-infection via an IL-12 driven mechanism	Expanded ESAT-6-specific T_{reg} cells express CD25, CTLA-4, GITR, CD103, ICOS, suggesting that they are activated and immune-suppressive.	[65]
		CD4+FoxP3+/Mtb-specific FoxP3+ T_{reg} cells expand in pulmonary lymph node and lungs by day 21 post-infection.	Depletion of FoxP3+ T_{regs} reduces CFU in lungs and spleen at day 23. Adoptive transfer of T_{reg}cells delays accumulation of Mtb-specific T_{eff} cells at day 21, implying that early T_{reg} expansion is detrimental.	[66,67]
		CD4+FoxP3+ T_{reg} cells expand in lungs and pulmonary lymph node of Mtb-infected mice starting from days 10 to 60 post-infection	Adoptive transfer of CD4+FoxP3+ T_{reg} cells along with T_{eff} cells into *Rag*$^{-/-}$ increase CFU burden by ~1 log in spleen and lungs compared to mice that receive only T_{eff} cells, implying that presence of T_{reg} can contribute to disease.	[68]
	Intranasal infection with *M. bovis* BCG Pasteur	Expansion of CD25+FoxP3+ T_{reg} cells in the lungs by days 21–25 post-infection.	Depletion of T_{reg} increases frequency of mycobacteria-specific IL-2+/IFN-γ+ cells but does not affect bacterial burden.	[69]
	Mtb H37Rv, Mtb Kurono, Mtb Erdman and *Mycobacterium bovis* BCG Tokyo aerosol-infected mice	T_{reg} frequencies were not determined	Depletion of T_{reg} reduces CFU burden at 21 days but not at 35 days post-infection, implying early T_{reg} expansion is detrimental. Also, CD4+CD25+ T_{reg} cells from chronically BCG or Mtb-infected mice fail to suppress PPD-induced proliferation and IFN-γ expression.	[70]
	Mtb H37Rv intravenous infection in TB-sensitive C3HeB/FeJ and TB-resistant C3H/HeN	CD25+FoxP3+ T_{reg} frequencies are higher in C3H/HeN compared to C3HeB/FeJ mice. Administration of heat-killed environmental mycobacteria *M. maserensis* boosts T_{reg} frequencies in C3HeB/FeJ mice.	Boosting T_{reg} frequencies by heat-killed *M. maserensis* decreases TB-induced lung pathology and improves survival in C3HeB/FeJ mice.	[71]
	Mtb H37Rv aerosol infection in TB-resistant C57BL/6JCit and TB-sensitive I/St mice	CD25+FoxP3+ T_{reg} frequencies are higher in B6 mice compared to I/St mice	B6 have fewer activated CD25+FoxP3− Th cells and reduced pathology compared to TB-sensitive I/St mice.	[72]
Non-human primate	Cynomolgous macaques infected with Mtb Erdman by bronchoscopic instillation.	CD4+FoxP3+ T_{reg} and total CD4+ cells are higher in Mtb- positive compared to -negative lung and lymph node tissue autopsied from infected animals. Macaques with latent TB have higher peripheral T_{reg} frequencies at baseline and 8 weeks post-infection compared to animals with active TB. Peripheral T_{reg} frequencies increase in active TB macaques from weeks 10 to 28 post-infection.	PHA and culture filtrate protein (CFP)-induced proliferation of PBMC was lower at baseline and 6 weeks post-infection in latent TB compared to active TB macaques, suggesting higher T_{reg} frequencies in the former and also that higher T_{reg} frequencies is a predisposing factor to acquisition of latent rather than active TB.	[73]
		IL-2 administration in early infection expands CD4+CD25+FoxP3+, CD8+CD25+FoxP3+ T_{reg} and CD4+ and CD8+ effector cells.	Administration of IL-2 reduced TB-induced inflammation and lesions, suggesting that IL-2 expanded effector as well T_{reg} cells contribute to anti-TB immunity.	[74]

Table 3. (Continued)

	Study model	Findings: T_{reg} frequency	Findings: T_{reg} function	Refs
Human	PBMC from healthy latent TB negative and active TB subjects. Cells from sites of TB infection (pleural, ascitic and pericardial fluids) analyzed	Increased frequencies of CD4+CD25+ T_{regs} in peripheral blood compared to healthy controls. Also, in TB T_{reg} are higher at sites of infection compared to peripheral blood.	Depletion of CD4+CD25+ cells from PBMC cultures from TB subjects results in higher Mtb-specific IFN-γ production implying presence of functional T_{regs}.	[75]
		Increased frequency of CD4+CD25+FoxP3+ T_{reg} cells in peripheral blood and pleural fluid of TB patients compared to healthy donors.		[76]
		Frequencies of peripheral CD4+CD25+$^{/high}$CTLA-4+ and CD4+CD25+$^{/high}$FoxP3+ are higher in TB compared to healthy controls.	CD4+CD25+$^{/high}$ T_{reg} cells can suppress BCG-induced IFN-γ production in *in-vitro* cultures of PBMC from TB patients, implying presence of functional T_{regs}.	[77]
	Sputum culture-positive and -negative MDR-TB subjects	Mtb-specific CD69−CD127−CD25^{high}FoxP3+Ki67+HLA-DR− T_{reg} frequencies are higher in sputum culture-positive (higher bacterial burden) than -negative patients.	T_{reg} function not studied	[78]
	PBMC from pulmonary TB and tuberculin+ subjects analyzed	No significant difference in frequencies of peripheral PPD-specific CD4+CD25+FoxP3+ T_{reg} cells in TB patients and controls.		[79]
	PBMC from latent (quantiferon positive) TB; pulmonary TB; pulmonary TB after treatment and healthy quantiferon-negative adults analyzed	Higher peripheral CD4+CD25+$^{/high}$ cells in TB patients. No significant difference in frequencies of peripheral CD4+CD25+CD127^{low}FoxP3+ T_{reg} between clinical categories studied.		[80]
		No significant difference in frequencies of peripheral CD4+CD45RA−CD25+, CD4+CD25+CD45RA−CD127^{low} and CD4+CD25+CD45RA−CD127^{low}FoxP3+ T_{reg} cells.	Suppression of proliferation of CD4+ effector T cells by CD45RA−CD4+CD25+ and CD45RA−CD4+CD25+CD127^{low} T_{regs} is dampened in active TB.	[64]

T_{reg} = regulatory T cell; STAT-3 = signal transducer and activator of transcription-3; Mtb = *Mycobacterium tuberculosis*; ESAT-6 = early secretory antigenic target-6; FoxP3 = forkhead box protein 3; IL = interleukin; PBMC = peripheral blood mononuclear cells ; CTLA-4 = cytotoxic T lymphocyte antigen 4; PPD = purified protein derivative; PHA = phytohaemagglutinin; CFU = colony-forming units; BCG = Bacille Calmette–Guérin; MDR-TB = multi-drug-resistant tuberculosis.

14 days post-infection, suggesting that the presence of T_{reg} cells hinders the appearance of protective Th1 responses [69]. Also, adoptive transfer of $CD25^+$ T_{reg} into Mtb-infected mice leads to reduced frequencies of Mtb-specific T_{eff} cells in the lungs at 14–17 days post-aerosol infection [66]. Importantly, the absence of protective Th1 responses due to expansion of T_{regs} leads to increased bacterial burden in the acute phase [67]. However, this dampening effect of T_{regs} on protective immune responses is transient and not evident in later stages of infection [65,69,70]. Depletion of $CD25^+$ T_{regs} had no effect on CFU burden or lung pathology in BCG or Mtb Erdman-infected mice at days 21 and 44 post-infection [69]. Similar results were reported in another study, where T_{reg} 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 [70]. It is important to note that depletion of T_{reg}using anti-CD25 as described above [69,70] has the disadvantage of also depleting activated $CD25^+$ T_{eff} cells. Nevertheless, studies carried out in mice using approaches of T_{reg} depletion [67,69,70] and adoptive transfer [66,68] have demonstrated that T_{reg} cells expand in the early phase of Mtb infection. However, it has also been now demonstrated in mice that Mtb-specific T_{regs} which expand early during infection are culled via IL-12 driven expression of T-bet by 32 days post-infection, T-bet being known for its pro-apoptotic effects [65]. How Mtb infection drives this early expansion of Mtb-specific T_{regs}, which is beneficial to the pathogen, remains to be elucidated.

Chronic phase: animal model studies show loss in T_{reg} frequency or failure to recruit T_{regs} to site of infection can be detrimental

In contrast to the role of T_{regs} in dampening protective Th responses in the early/acute stage of infection in murine models, several studies in mouse and primate models highlight that T_{regs} might be potentially beneficial by regulating excessive inflammation 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 T_{reg} frequencies and consequently less TB-induced lung pathology in the chronic phase of the disease [71,72] compared to TB-sensitive mice, which recruit significantly fewer T_{regs} to the lung [72]. Interestingly, oral administration of heat-killed *M. maserensis* (environmental mycobacterium) in TB-sensitive C3HeN/FeJ mice resulted in a boost in T_{reg} frequencies with a reduction in lung pathology and improved survival [71]. 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 [73]. In this experimental system it was observed that macaques that developed latent TB had higher basal pre-infection T_{reg} frequencies compared to animals that develop active disease [73]. In a separate study, IL-2 administered either pre- or post-Mtb infection in macaques resulted in T_{reg} expansion which, in turn, led to reduced bacterial burden and TB-induced pathology, suggesting that expansion of T_{reg} cells in the later stage of chronic TB infection can help to control excessive TB-induced inflammation [74].

Human studies

In contrast to animal model studies, where changes in circulating T_{reg} frequency can impact infection levels, reports of T_{reg} frequencies in human TB are varied. Some studies show an increase in peripheral T_{reg} frequencies in TB [75–78]. However, our study [64] and others [79,80] found no differences in peripheral T_{reg} frequencies between pulmonary TB patients and healthy controls. This disparity may be linked to differences in markers used for T_{reg} delineation, which vary and can include CD4 and CD25 [75]; CD4, CD25 and FoxP3 [76,79], a combination of CD4, CD45RA/CD45RO, CD127, CD25 and FoxP3 to identify memory T_{regs} [80,64] or $CD4^+CD127^{low}CD25^+FoxP3^+CD45RO^+Ki67^+$ to identify activated T_{reg} cells [80]. Beyond variation in markers used for definition, a further limitation of only tracking T_{reg} frequency to define T_{reg} function in a disease such as TB is the impact of trafficking; thus, T_{reg} 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 [75,86].

Chronic phase: novel qualitative studies of T_{reg} function in humans highlight emergence of T_{reg}-resistant T effectors in chronic TB

Many studies have probed T_{reg} frequency; however, few human or animal model studies have analyzed qualitative aspects of T_{reg} function in TB. Some studies have shown that T_{reg} cells from pulmonary TB patients retain their capacity to suppress autologous T_{eff} cells [87–89]. Data from our laboratory show that autologous suppression mediated by $CD4^+CD45RA^-CD25^+CD127^{low}$ memory T_{reg} cells isolated from subjects with pulmonary TB in south India is significantly compromised [64]. By testing isolated T_{regs} from healthy controls on T_{eff} isolated from TB subjects and vice versa, we demonstrated that this impairment is not due to the loss of suppressive potential of T_{reg} cells isolated from TB subjects; instead, it is due to the effector cells from TB subjects acquiring resistance to T_{reg}-mediated suppression [64]. Thus, $CD127^{low}CD25^+$ T_{reg} cells from TB subjects were effective in suppressing T_{eff} from healthy controls but not those from TB subjects; conversely, T_{reg}

isolated from healthy controls effectively suppressed autologous T_{eff} but failed to suppress T_{eff} from TB subjects [64]. Phenotypical analysis of the T_{reg}-resistant T_{eff} 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-DR$^+$ subset, in particular, restored sensitivity of HLA-DR$^-$ T_{eff} to autologous T_{reg} suppression, thereby confirming that resistance of T_{eff} from TB subjects to T_{reg}-mediated suppression was due to the presence of HLA-DR$^+$ cells [64]. The expansion of HLA-DR$^+$CD4$^+$ T cells in TB is driven by infection, as anti-tubercular (anti-TB) treatment reduced the frequencies of HLA-DR$^+$CD4$^+$ T cells [64,90,91]; indeed, we have shown that by dampening the frequency of HLA-DR$^+$ cells, anti-TB treatment restores T_{eff} cell sensitivity to autologous T_{reg} cell-mediated suppression [64]. Consequently, measuring HLA-DR$^+$CD4$^+$ T cell frequency can potentially be used to monitor treatment responses and predict efficacy of treatment [91]. In this context, our observation that HLA-DR$^+$CD4$^+$ T_{eff} cells resistant to T_{reg}-mediated suppression provides a mechanistic basis for how the expansion of HLA-DR$^+$ T effectors may be detrimental in TB [64]. The findings from our study are summarized in Fig. 2.

This observation of the emergence of T_{reg}-resistant T_{eff} 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 T_{reg}-mediated suppression and their depletion restores suppression in *in-vitro* cultures [61]. A similar phenomenon of the emergence of suppression resistant effectors has been reported in systemic lupus erythromatosis [92], multiple sclerosis [93], type 1 diabetes [59,94] and juvenile idiopathic arthritis [95], with potentially varying mechanisms underpinning such resistance. In multiple sclerosis, it was attributed to high T_{eff} cell-derived granzyme B [93]; in type 1 diabetes due to down-regulation of TGF-βRII on T_{eff} cells and consequently reduced TGF-β-mediated suppression [94] and

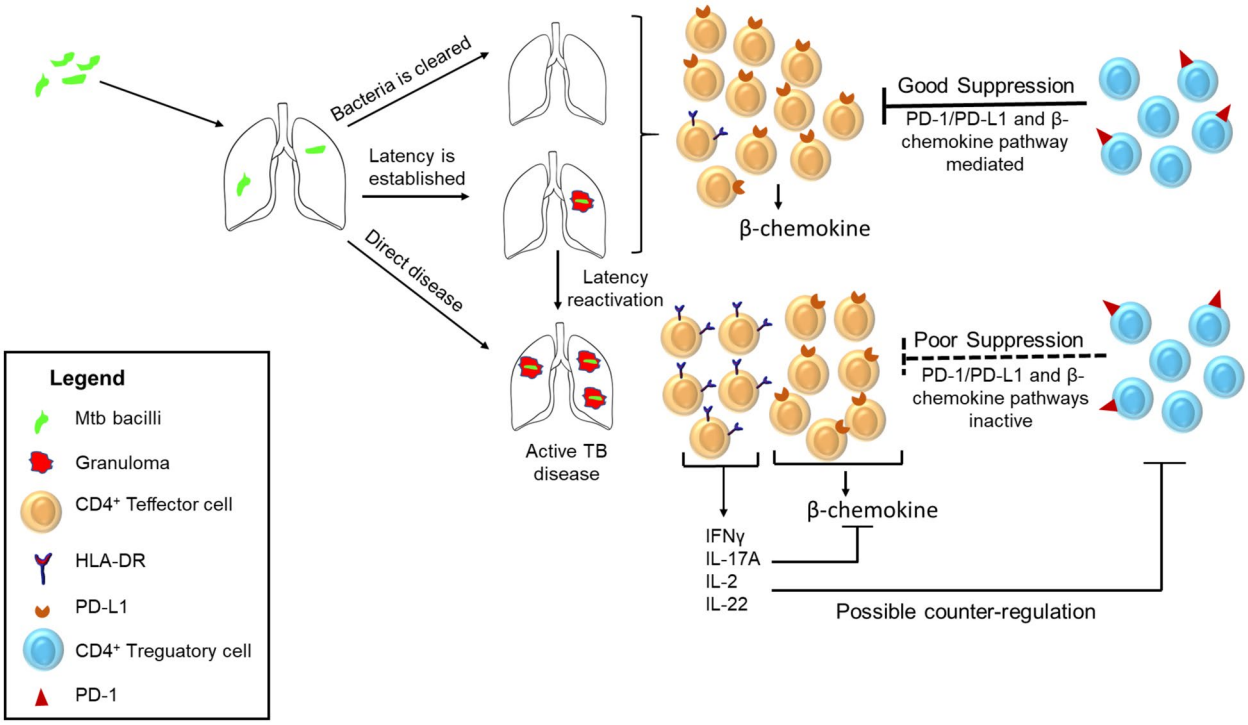


Fig. 2. A diagrammatic model which highlights the difference in T_{reg} suppression in healthy, latently infected individuals and active TB subjects in context of expansion of HLA-DR$^+$CD4$^+$ memory T cells. Individuals infected with TB can either clear the bacteria, become latently infected or develop 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 such as 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 T_{reg} suppression is good. However, in active TB, HLA-DR$^+$CD4$^+$ T cells expand and T_{reg}-mediated suppression becomes poor. The T_{reg} 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, IFN-γ and IL-22 that are secreted by the expanded HLA-DR$^+$CD4$^+$ T cells.

in juvenile idiopathic arthritis, as in TB [64], due to expansion of activated CD69$^+$HLA-DR$^+$ T_{eff} cells which were T_{reg} suppression-resistant [95].

Mechanisms that underpin T_{reg} dysregulation in TB

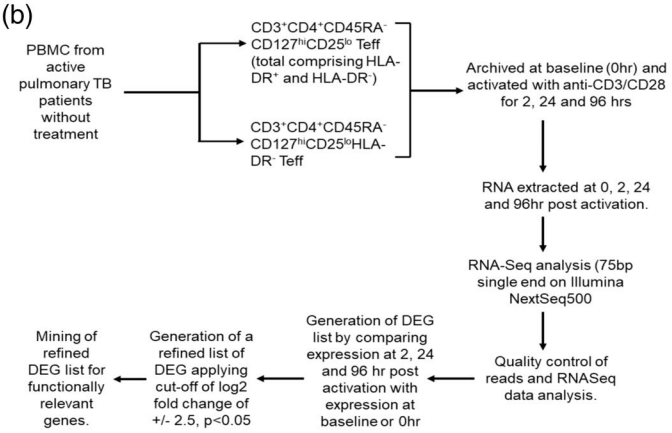
Evidence for altered expression of cell surface molecules on T_{eff} that are important for engaging with T_{reg} cells

T_{reg} cells suppress via a variety of contact dependent and independent mechanisms (Fig. 1). Some of the key molecules shown to be involved in promoting T_{reg} suppression include CTLA-4, PD-1, CD39, CD73, PD-L1, LAG3, etc. Several lines of data show some of these molecules, e.g. PD-1, PD-L1, CD39, CD73 and LAG3, are altered in TB. PD-1 and PD-L1 expression is elevated in CD4$^+$ T cells from TB subjects compared to healthy controls [96]; CD39 and CD73 are increased in lung parenchymal CD4$^+$ T cells of Mtb-infected mice [97] and LAG3 is increased in granuloma of macaques with active TB compared to those with latent TB [98]. However, the function of these molecules has not been specifically studied in the context of T_{reg} suppression in TB. Our study of transcriptomic and functional analysis of cells from pulmonary TB patients provides evidence for a role for PD-1/PD-L1 and β chemokine/C-C chemokine receptor type 5 (CCR5) interactions [64]. We identified that in TB the expanded subset of HLA-DR$^+$ T_{eff} cells is resistant to T_{reg}-mediated suppression while HLA-DR$^-$ T_{eff} cells remain sensitive; a cellular population comprising both these subsets (HLA-DR$^+$ and HLA-DR$^-$) was rendered resistant to T_{reg}-mediated suppression, indicating the function of HLA-DR$^+$ effectors to be dominant in this mixture [64]. To probe the underlying mechanisms, we captured HLA-DR$^-$ and total (HLA-DR$^+$ and HLA-DR$^-$) cell fractions from subjects with confirmed treatment-naive pulmonary TB [diagnosed by presence of acid-fast bacilli (AFB) in sputum and Genexpert positivity (Fig. 3a)] and analysed the transcriptome of these cells through RNA sequence analysis over time

(a)

Donor	Age	Gender	Sputum AFB	Genexpert MTB	TB diagnosis
1	60	M	Positive	Not detected	Positive
2	55	M	Positive	Not detected	Positive
3	30	M	Positive	Detected Low	Positive
4	26	M	Positive	Detected High	Positive
5	48	M	Positive	Detected Medium	Positive

(b)



(c)

		Total Teff			HLA-DR- Teff		
		2h	24h	96h	2h	24h	96h
Cytokines and Chemokines	IL2	9.62618	8.95558				
	IL17A	2.78464					
	CSF2	10.6616	9.79965	8.45831			
	IL22	4.53458		2.98718			
	IFNγ	5.46465	3.94531		5.80443	3.7734	
	TNFα	6.01587	3.75848		5.77639		
	LTA/TNFβ	5.00325	8.17732	5.58176		8.19545	
	CCL3L3				7.60568		
	CCL3	5.74792	6.77851	5.61295	6.23579	7.43753	5.43272
	CCL4		2.8932		3.3452	3.63758	
	TGFβ		-5.3666	-4.45233		-5.61617	-4.88765
Cell surface activation markers	CD40LG	3.95782			3.90243		
	IL2RA/CD25		3.9596	7.92118		7.13143	7.77332
	CD38						2.92012
	HLA-DRA			3.70296			2.51047
	HLA-DRB			3.88552			2.62443
Other cell surface markers	CD46				5.94787		
	CD164				5.83707		
	CD274				6.25015		
	TNFSF10/TRAIL				5.97646		
	TRAF1	2.60691	-3.05364				
	TRAF3	2.70563					
	FASLG	4.89282					
	SEMA7A	6.14134	6.51356			5.5936	3.07648
	TNFRSF8/CD30			9.18873			
Transcription factors	FOXP3		3.40188	5.26935		3.58261	4.56951
	EOMES				2.74817		
Marker for proliferation	TYMS		5.58962	9.82266		7.33982	9.96018

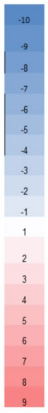


Fig. 3. T_{reg} suppression resistant total T_{eff} and T_{reg} suppression sensitive HLA-DR$^-$ T_{eff} cells have distinct expression patterns with respect to certain cytokines and cell surface receptors. (a) A brief summary of clinical details of treatment naive pulmonary tuberculosis (TB) donors, including sputum acid fast bacilli (AFB) and Genexpert test results. (b) An outline of methodology used for sorting and archiving of total and HLA-DR$^+$-depleted (HLA-DR$^-$) T_{eff} cell populations for RNA-Seq analysis, as described previously [64]. Briefly, total T_{eff} (comprising HLA-DR$^+$ and HLA-DR$^-$ cells) and HLA-DR$^-$ Teff were sorted by flow cytometry from 5 pulmonary TB patients. RNA was isolated from both cell fractions at 0, 2, 24 and 96 h post-activation with anti-CD3/CD28 mitogenic beads and subjected to sequencing using the Illumina NEXTSeq 500 platform (see [64]). Activation-induced longitudinal changes in gene expression was first determined relative to the unstimulated control using a cut-off of $P < 0{\cdot}05$, $\log_2$ fold change (FC). Next, genes differentially expressed with time were compared between the total (T_{reg}-resistant) *versus* HLA-DR$^-$ (T_{reg}-sensitive) T_{eff} cells. (c) The database of essential genes (DEG) list was mined for genes implicated in T cell function. A summary of these results is shown. The numbers in boxes denote $\log_2$ FC for expression at 2, 24 and 96 h compared to unactivated cells at baseline for each cell fraction. For further details on procedure and complete DEG list please see reference 64.

post-activation with anti-CD3/anti-CD28 (Fig. 3b). Gene expression analysis of each cell fraction post-activation relative to the unstimulated control allowed identification of longitudinal changes, while comparison of the two fractions highlighted differentially expressed genes [64]. Of the 193 and 89 differentially expressed genes identified in the HLA-DR$^-$ and total T_{eff} fractions, respectively, at 2 h post activation, elevated expression of PD-L1 and β-chemokines were noted to be significantly elevated in HLA-DR$^-$ T_{reg}-sensitive T_{eff} (Fig. 3c). Through blocking studies, we confirmed the functional significance of both pathways in T_{reg}-mediated suppression of HLA-DR$^-$ T effectors, pointing to the importance of these pathways in maintaining T_{reg}-mediated homeostasis in TB [64]. Our findings are summarized in Fig. 2. PD-1/PD-L1 interactions and β-chemokine/CCR5 interactions have been previously implicated in promoting T_{reg}-mediated suppression [99,100]. C-C motif chemokine ligand (CCL)3 and CCL4 secreted by T_{regs} serve as chemoattractants for T_{eff} cells, and T_{eff} cells from mice deficient in CCL3 and CCL4 fail to migrate and conjugate with T_{reg} cells [100]. Moreover, T_{regs} from type 1 diabetes patients are deficient in CCL3 and CCL4, and this compromises their ability to suppress [100].

Apart from differences in PD-L1 and β-chemokine levels (Fig. 2), our transcriptome analysis of T_{reg}-sensitive HLA-DR$^-$ and suppression-resistant HLA-DR$^+$ cells identified several additional cell surface markers [CD46, TNF-related apoptosis inducing ligand (TRAIL), TNF receptor-associated factor (TRAF)1, TRAF3, FAS ligand (FASLG), CD30 and semaphorin 7A (SEMA7A)] (Fig. 3c), some of which have previously been implicated in T_{reg} function [101–104]. Engagement of complement receptor CD46 results in suppression of bystander CD4$^+$ T cells via an IL-10-dependent mechanism [101,102]. CD46 cross-linking also suppresses mycobacteria-specific CD4$^+$ T cell responses [105]. TRAIL is a regulator of T cell activation; its absence leads to autoimmunity and reduction in T_{reg} frequencies, while its presence dampens Th1 responses and boosts T_{regs} [103]. TRAF1 inhibits Th2 differentiation [106]; TRAF3 controls proximal T cell activation events and its absence in mice leads to elevated thymus derived T_{reg} cell frequencies [104]; FASLG is a marker for T cell activation and is expressed on Th1 cells [107,108]; and SEMA7A and CD30 have been implicated in Th1 and Th17 differentiation [109,110]. However, a role for these pathways impacting T_{eff} function in TB remains to be elucidated.

Evidence for exaggerated expression of cytokines that counterbalance T_{reg} function

It is well recognized that proinflammatory cytokines can suppress the generation and function of T_{reg} cells. By directly comparing the transcriptome of T_{reg}-resistant HLA-DR$^+$ effector CD4$^+$ T cells isolated from TB subjects with that of the T_{reg}-sensitive fraction depleted of HLA-DR$^+$ CD4$^+$ T cells, we provide the first evidence that HLA-DR$^+$ T_{effs} from TB express a number of proinflammatory cytokines, including IL-2, IFN-γ, colony stimulating factor-2 (CSF2), IL-17A and IL-22 ([64], Fig. 3c). This exaggerated cytokine profile was noted in T_{effs} stimulated with both Mtb antigen as well as polyclonal stimulation [64] and could be possibly responsible for counter-regulation of T_{reg}-mediated suppression pathways, as summarized in Fig. 2. Both IFN-γ and IL-17A, although crucial for Mtb control [111,112], are also recognized to counter-regulate T_{reg} development and function and their exaggerated expansion, therefore, could be one mechanism for T_{eff} resistance to T_{reg} control [113–115].

Signal strength

A third potentially important consideration in the mechanisms that underpin how activated, HLA-DR$^+$ T effectors become resistant to T_{reg} cells may be linked to the quality and strength of the primary signal that activates effector cells. The strength of the 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 [116,117]). Previous studies show that effectors activated by a very strong signal strength become refractory to suppression mediated by T_{reg} cell co-culture of human CD25$^-$ T_{eff} and autologous CD25$^+$ T_{reg} 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) [118]. 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 early secretory antigenic target 6 (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. antigen 85B (Ag85B), can confer greater protection [119]. 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 killer cell lectin-like receptor subfamily G member 1 (KLRG1), lower CCR7, CD127 and CD62L, compared to Ag85B-specific cells [119]. Whether T_{reg}-resistant HLA-DR$^+$ T_{eff} 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 T_{regs}, both nT_{reg} and antigen-specific, has several dimensions. While T_{regs} 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 [120,121]. The immune response in TB is clearly disordered, and the theme of balance between protective and pathogenic responses has been visited in the past [122]. In fact, a balance between Th1/Th17 and immune-regulatory responses is associated with better clearance of Mtb infection [123]. In this context, the expansion of HLA-DR$^+$ T_{eff} cells in TB is a probable marker for inflammation associated with enhanced disease risk [124]. It has now been demonstrated that this expanded subset exhibits resistance to suppression mediated by natural T_{reg} cells [64]. The putative role of proinflammatory cytokines (IFN-γ, IL-17A, IL-2, CSF2 and IL-22), β-chemokines and PD-1/PD-L1 interactions in modulating T_{eff} resistance to T_{reg} suppression in TB has been identified (Fig. 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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Disclosures

The authors declare that they have no competing interests.

References

1 Moran AE, Holzapfel KL, Xing Y *et al*. T cell receptor signal strength in Treg and iNKT cell development demonstrated by a novel fluorescent reporter mouse. J Exp Med 2011; **208**:1279–89.

2 Jordan MS, Boesteanu A, Reed AJ *et al*. Thymic selection of CD4$^+$CD25$^+$ regulatory T cells induced by an agonist self-peptide. Nat Immunol 2001; **2**:301–6.

3 Tai X, Cowan M, Feigenbaum L, Singer A. CD28 costimulation of developing thymocytes induces Foxp3 expression and regulatory T cell differentiation independently of interleukin 2. Nat Immunol 2005; **6**:152–62.

4 Nazzal D, Gradolatto A, Truffault F *et al*. Human thymus medullary epithelial cells promote regulatory T-cell generation by stimulating interleukin-2 production via ICOS ligand. Cell Death Dis 2014; **5**:e1420.

5 Watanabe N, Wang YH, Lee HK *et al*. Hassall's corpuscles instruct dendritic cells to induce CD4$^+$CD25$^+$ regulatory T cells in human thymus. Nature 2005; **436**:1181–5.

6 Fontenot JD, Gavin MA, Rudensky AY. Foxp3 programs the development and function of CD4+CD25+ regulatory T cells. Nat Immunol 2003; **4**:330–6.

7 Brunkow ME, Jeffery EW, Hjerrild KA *et al*. Disruption of a new forkhead/winged-helix protein, scurfin, results in the fatal lymphoproliferative disorder of the scurfy mouse. Nat Genet 2001; **27**:68–73.

8 Gavin MA, Rasmussen JP, Fontenot JD *et al*. Foxp3-dependent programme of regulatory T-cell differentiation. Nature 2007; **445**:771–5.

9 Lin W, Haribhai D, Relland LM *et al*. Regulatory T cell development in the absence of functional Foxp3. Nat Immunol 2007; **8**:359–68.

10 Wan YY, Flavell RA. Regulatory T-cell functions are subverted and converted owing to attenuated Foxp3 expression. Nature 2007; **445**:766–70.

11 Zorn E, Nelson EA, Mohseni M *et al*. IL-2 regulates FOXP3 expression in human CD4+CD25+ regulatory T cells through a STAT-dependent mechanism and induces the expansion of these cells *in vivo*. Blood 2006; **108**:1571–9.

12 Tone Y, Furuuchi K, Kojima Y *et al*. Smad3 and NFAT cooperate to induce Foxp3 expression through its enhancer. Nat Immunol 2008; **9**:194–202.

13 Burchill MA, Yang J, Vogtenhuber C *et al*. IL-2 receptor beta-dependent STAT5 activation is required for the development of Foxp3$^+$ regulatory T cells. J Immunol 2007; **178**: 280–90.

14 Feske S, Müller JM, Graf D *et al*. Severe combined immunodeficiency due to defective binding of the nuclear factor of activated T cells in T lymphocytes of two male siblings. Eur J Immunol 1996; **26**:2119–26.

15 Sauer S, Bruno L, Hertweck A *et al*. T cell receptor signaling controls Foxp3 expression via PI3K, Akt, and mTOR. Proc Natl Acad Sci USA 2008; **105**:7797–802.

16 Chen W, Jin W, Hardegen N *et al*. Conversion of peripheral CD4+CD25− naive T cells to CD4$^+$CD25$^+$ regulatory T cells by TGF-β induction of transcription factor Foxp3. J Exp Med 2003; **198**:1875–86.

17 Horwitz DA, Zheng SG, Wang J, Gray JD. Critical role of IL-2 and TGF-beta in generation, function and stabilization of Foxp3$^+$CD4$^+$ Treg. Eur J Immunol 2008; **38**:912–5.

18 Semple K, Nguyen A, Yu Y *et al*. Strong CD28 costimulation suppresses induction of regulatory T cells from naive precursors through Lck signaling. Blood 2011; **117**:3096–103.

19 Gottschalk RA, Corse E, Allison JP. TCR ligand density and affinity determine peripheral induction of Foxp3 *in vivo*. J Exp Med 2010; **207**:1701–11.

20 Kretschmer K, Apostolou I, Hawiger D *et al*. Inducing and expanding regulatory T cell populations by foreign antigen. Nat Immunol 2005; **6**:1219–27.

21 Zeng H, Zhang R, Jin B, Chen L. Type 1 regulatory T cells: a new mechanism of peripheral immune tolerance. Cell Mol Immunol 2015; **12**:566–71.

22 Groux H, O'Garra A, Bigler M *et al*. A $CD4^+$ T-cell subset inhibits antigen-specific T-cell responses and prevents colitis. Nature 1997; **389**:737–42.

23 Gagliani N, Magnani CF, Huber S *et al*. Co-expression of CD49b and LAG-3 identifies human and mouse T regulatory type 1 cells. Nat Med 2013; **19**:739–46.

24 Jin JO, Han X, Yu Q. Interleukin-6 induces the generation of IL-10-producing Tr1 cells and suppresses autoimmune tissue inflammation. J Autoimmun 2013; **40**:28–44.

25 Pot C, Jin H, Awasthi A *et al*. Cutting edge: IL-27 induces the transcription factor c-Maf cytokine IL-21, and the costimulatory receptor ICOS that coordinately act together to promote differentiation of IL-10-producing Tr1 cells. J Immunol 2009; **183**:797–801.

26 Levings MK, Gregori S, Tresoldi E *et al*. Differentiation of Tr1 cells by immature dendritic cells requires IL-10 but not $CD25^+CD4^+$ Tr cells. Blood 2005; **105**:1162–9.

27 Yang Y, Ochando J, Yopp A *et al*. IL-6 plays a unique role in initiating c-Maf expression during early stage of CD4 T cell activation. J Immunol 2005; **174**:2720–9.

28 Wang H, Meng R, Li Z *et al*. IL-27 induces the differentiation of Tr1-like cells from human naive $CD4^+$ T cells via the phosphorylation of STAT1 and STAT3. Immunol Lett 2011; **136**:21–8.

29 Joller N, Lozano E, Burkett PR *et al*. Treg cells expressing the coinhibitory molecule TIGIT selectively inhibit proinflammatory Th1 and Th17 cell responses. Immunity 2014; **40**:569–81.

30 Collison LW, Workman CJ, Kuo TT *et al*. The inhibitory cytokine IL-35 contributes to regulatory T-cell function. Nature 2007; **450**:566–9.

31 Wu X, Tian J, Wang S. Insight into non-pathogenic Th17 cells in autoimmune diseases. Front Immunol 2018; **9**:1112.

32 Hashemi V, Farrokhi AS, Tanomand A *et al*. Polymorphism of Foxp3 gene affects the frequency of regulatory T cells and disease activity in patients with rheumatoid arthritis in Iranian population. Immunol Lett 2018; **204**:16–22.

33 Rossetti M, Spreafico R, Consolaro A *et al*. TCR repertoire sequencing identifies synovial Treg cell clonotypes in the bloodstream during active inflammation in human arthritis. Ann Rheum Dis 2017; **76**:435–41.

34 Zare HR, Habibagahi M, Vahdati A, Habibagahi Z. Patients with active rheumatoid arthritis have lower frequency of nTregs in peripheral blood. Iran J Immunol 2015; **12**:166–75.

35 Beavis PA, Gregory B, Green P *et al*. Resistance to regulatory T cell-mediated suppression in rheumatoid arthritis can be bypassed by ectopic Foxp3 expression in pathogenic synovial T cells. Proc Natl Acad Sci USA 2011; **108**:16717–22.

36 Haas J, Hug A, Viehöver A *et al*. Reduced suppressive effect of $CD4^+CD25^{high}$ regulatory T cells on the T cell immune response against myelin oligodendrocyte glycoprotein in patients with multiple sclerosis. Eur J Immunol 2005; **35**:3343–52.

37 Azimi M, Ghabaee M, Moghadasi AN *et al*. Immunomodulatory function of Treg-derived exosomes is impaired in patients with relapsing-remitting multiple sclerosis. Immunol Res 2018; **66**:513–20.

38 Kimura K, Hohjoh H, Fukuoka M *et al*. Circulating exosomes suppress the induction of regulatory T cells via let-7i in multiple sclerosis. Nat Commun 2018; **9**:17.

39 Venken K, Hellings N, Broekmans T *et al*. Natural naive $CD4^+CD25^+CD127^{low}$ regulatory T cell (Treg) development and function are disturbed in multiple sclerosis patients: recovery of memory Treg homeostasis during disease progression. J Immunol 2008; **180**:6411–20.

40 Sambucci M, Gargano F, De Rosa V *et al*. FoxP3 isoforms and PD-1 expression by T regulatory cells in multiple sclerosis. Sci Rep 2018; **8**:3674.

41 Kato R, Sumitomo S, Tsuchida Y *et al*. $CD4^+CD25^+LAG3^+$ T cells with a feature of Th17 cells associated with systemic lupus erythematosus disease activity. Front Immunol 2019; **10**:1619.

42 Jin X, Zhang C, Gong L *et al*. Altered expression of CD39 on memory regulatory T cells in type 1 diabetes patients. J Diabetes 2019; **11**:440–8.

43 Scherm MG, Serr I, Zahm AM *et al*. miRNA142-3p targets Tet2 and impairs Treg differentiation and stability in models of type 1 diabetes. Nat Commun 2019; **10**:5697.

44 Hope CM, Welch J, Mohandas A *et al*. Peptidase inhibitor 16 identifies a human regulatory T-cell subset with reduced FOXP3 expression over the first year of recent onset type 1 diabetes. Eur J Immunol 2019; **49**:1235–50.

45 Kurup SP, Obeng-Adjei N, Anthony SM *et al*. Regulatory T cells impede acute and long-term immunity to blood-stage malaria through CTLA-4. Nat Med 2017; **23**:1220–5.

46 Boyle MJ, Jagannathan P, Farrington LA *et al*. Decline of $FoxP3^+$ Regulatory CD4 T cells in peripheral blood of children heavily exposed to malaria. PLOS Pathog 2015; **11**:e1005041.

47 Tillu H, Tripathy AS, Reshmi PV, Cecilia D. Altered profile of regulatory T cells and associated cytokines in mild and moderate dengue. Eur J Clin Microbiol Infect Dis 2016; **35**:453–61.

48 Jayaratne HE, Wijeratne D, Fernando S *et al*. Regulatory T-cells in acute dengue viral infection. Immunology 2018; **154**:89–97.

49 Roider J, Ngoepe A, Muenchhoff M *et al*. Increased regulatory T-cell activity and enhanced T-cell homeostatic signaling in slow progressing HIV-infected children. Front Immunol 2019; **10**:213.

50 Eggena MP, Barugahare B, Jones N *et al*. Depletion of regulatory T cells in HIV infection is associated with immune activation. J Immunol 2005; **174**:4407–14.

51 Chase AJ, Yang HC, Zhang H *et al*. Preservation of $FoxP3^+$ regulatory T cells in the peripheral blood of human

immunodeficiency virus type 1-infected elite suppressors correlates with low CD4+ T-cell activation. J Virol 2008; **82**:8307–15.

52 Xiao J, Zhang L, Dong Y *et al*. PD-1 Upregulation is associated with exhaustion of regulatory T cells and reflects immune activation in HIV-1-infected individuals. AIDS Res Hum Retroviruses 2019; **35**:444–52.

53 Younes SA, Talla A, Pereira Ribeiro S *et al*. Cycling CD4+ T cells in HIV-infected immune non-responders have mitochondrial dysfunction. J Clin Invest 2018; **128**:5083–94.

54 Whibley N, Maccallum DM, Vickers MA *et al*. Expansion of Foxp3+ T-cell populations by *Candida albicans* enhances both Th17-cell responses and fungal dissemination after intravenous challenge. Eur J Immunol 2014; **44**:1069–83.

55 Pandiyan P, Conti HR, Zheng L *et al*. CD4+CD25+Foxp3+ regulatory T cells promote Th17 cells *in vitro* and enhance host resistance in mouse *Candida albicans* Th17 cell infection model. Immunity 2011; **34**:422–34.

56 Kumar P, Misra P, Thakur CP *et al*. T cell suppression in the bone marrow of visceral leishmaniasis patients: impact of parasite load. Clin Exp Immunol 2018; **191**:318–27.

57 Bhattacharya P, Ghosh S, Ejazi SA *et al*. Induction of IL-10 and TGFβ from CD4+CD25+FoxP3+ T cells correlates with parasite load in Indian kala-azar patients infected with *Leishmania donovani*. PLOS Negl Trop Dis 2016; **10**:e0004422.

58 Ihantola EL, Viisanen T, Gazali AM *et al*. Effector T cell resistance to suppression and STAT3 signaling during the development of human type 1 diabetes. J Immunol 2018; **201**:1144–53.

59 Lawson JM, Tremble J, Dayan C *et al*. Increased resistance to CD4+CD25hi regulatory T cell-mediated suppression in patients with type 1 diabetes. Clin Exp Immunol 2008; **154**:353–9.

60 Schneider A, Rieck M, Sanda S *et al*. The effector T cells of diabetic subjects are resistant to regulation via CD4+FOXP3+ regulatory T cells. J Immunol 2008; **181**:7350–5.

61 Basdeo SA, Moran B, Cluxton D *et al*. Polyfunctional, Pathogenic CD161+ Th17 lineage cells are resistant to regulatory t cell-mediated suppression in the context of autoimmunity. J Immunol 2015; **195**:528–40.

62 Thorborn GS, Pomeroy L, Ishohanni H *et al*. Elevated effector cell sensitivity to Treg-cell suppression that is not associated with reduced Th17-cell expression distinguishes HIV+ asymptomatic subjects from progressors. Eur J Immunol 2012; **42**:138–46.

63 Elahi S, Dinges WL, Lejarcegui N *et al*. Protective HIV-specific CD8+ T cells evade Treg cell suppression. Nat Med 2011; **17**:989–95.

64 Ahmed A, Adiga V, Nayak S *et al*. Circulating HLA-DR+CD4+ effector memory T cells resistant to CCR5 and PD-L1 mediated suppression compromise regulatory T cell function in tuberculosis. PLOS Pathog 2018; **14**:e1007289.

65 Shafiani S, Dinh C, Ertelt JM *et al*. Pathogen-specific Treg cells expand early during *Mycobacterium tuberculosis* infection but are later eliminated in response to Interleukin-12. Immunity 2013; **38**:1261–70.

66 Shafiani S, Tucker-Heard G, Kariyone A *et al*. Pathogen-specific regulatory T cells delay the arrival of effector T cells in the lung during early tuberculosis. J Exp Med 2010; **207**: 1409–20.

67 Scott-Browne JP, Shafiani S, Tucker-Heard G *et al*. Expansion and function of Foxp3-expressing T regulatory cells during tuberculosis. J Exp Med 2007; **204**:2159–69.

68 Kursar M, Koch M, Mittrücker HW *et al*. Cutting edge: regulatory T cells prevent efficient clearance of *Mycobacterium tuberculosis*. J Immunol 2007; **178**:2661–5.

69 Quinn KM, McHugh RS, Rich FJ *et al*. Inactivation of CD4+CD25+ regulatory T cells during early mycobacterial infection increases cytokine production but does not affect pathogen load. Immunol Cell Biol 2006; **84**:467–74.

70 Ozeki Y, Sugawara I, Udagawa T *et al*. Transient role of CD4+CD25+ regulatory T cells in mycobacterial infection in mice. Int Immunol 2010; **22**:179–89.

71 Cardona P, Marzo-Escartín E, Tapia G *et al*. Oral administration of heat-killed *Mycobacterium manresensis* delay progression toward active tuberculosis in C3HeB/FeJ mice. Front Microbiol 2016; **6**:1482.

72 Kapina MA, Rubakova EI, Majorov KB *et al*. Capacity of lung stroma to educate dendritic cells inhibiting mycobacteria-specific T-cell response depends upon genetic susceptibility to tuberculosis. PLOS ONE 2013; **8**:e72773.

73 Green AM, Mattila JT, Bigbee CL *et al*. CD4+ regulatory T cells in a cynomolgus macaque model of *Mycobacterium tuberculosis* infection. J Infect Dis 2010; **202**:533–41.

74 Chen CY, Huang D, Yao S *et al*. IL-2 simultaneously expands Foxp3+ T regulatory and T effector cells and confers resistance to severe tuberculosis (TB): implicative Treg-T effector cooperation in immunity to TB. J Immunol 2012; **188**: 4278–88.

75 Guyot-Revol V, Innes JA, Hackforth S *et al*. Regulatory T cells are expanded in blood and disease sites in patients with tuberculosis. Am J Respir Crit Care Med 2006; **173**:803–10.

76 Chen X, Zhou B, Li M *et al*. CD4+CD25+FoxP3+ regulatory T cells suppress *Mycobacterium tuberculosis* immunity in patients with active disease. Clin Immunol 2007; **123**: 50–9.

77 Li L, Lao SH, Wu CY. Increased frequency of CD4+CD25high Treg cells inhibit BCG-specific induction of IFN-gamma by CD4+ T cells from TB patients. Tuberculosis 2007; **87**: 526–34.

78 Ferrian S, Ross M, Conradie F *et al*. Frequency of circulating CD4+Ki67+HLA-DR- T regulatory cells prior to treatment for multidrug resistant tuberculosis can differentiate the severity of disease and predict time to culture conversion. Front Immunol 2018; **9**:2438.

79 Ghazalsofala R, Rezaee SA, Rafatpanah H *et al*. Evaluation of CD4+CD25+ FoxP3+ regulatory T cells and FoxP3 and CTLA-4 gene expression in patients with newly diagnosed tuberculosis in northeast of Iran. Jundishapur J Microbiol 2015; **8**:e17726.

80 Zewdie M, Howe R, Hoff ST *et al*. *Ex-vivo* characterization of regulatory T cells in pulmonary tuberculosis patients, latently infected persons, and healthy endemic controls. Tuberculosis 2016; **100**:61–8.

81 Drain PK, Bajema KL, Dowdy D *et al*. Incipient and subclinical tuberculosis: a clinical review of early stages and progression of infection. Clin Microbiol Rev 2018; **31**:e00021–e118.

82 Ahmed A, Rakshit S, Vyakarnam A. HIV-TB co-infection: mechanisms that drive reactivation of *Mycobacterium tuberculosis* in HIV infection. Oral Dis 2016; **22**(Suppl 1):53–60.

83 Queval CJ, Brosch R, Simeone R. The macrophage: a disputed fortress in the battle against *Mycobacterium tuberculosis*. Front Microbiol 2017; **8**:2284.

84 Ramakrishnan L. Revisiting the role of the granuloma in tuberculosis. Nat Rev Immunol 2012; **12**:352–66.

85 Ndlovu H, Marakalala MJ. Granulomas and inflammation: host-directed therapies for tuberculosis. Front Immunol 2016; **7**:434.

86 Semple PL, Binder AB, Davids M *et al*. Regulatory T cells attenuate mycobacterial stasis in alveolar and blood-derived macrophages from patients with tuberculosis. Am J Respir Crit Care Med 2013; **187**:1249–58.

87 Hougardy JM, Verscheure V, Locht C, Mascart F. *In vitro* expansion of $CD4^+CD25^{high}FOXP3^+CD127^{low}$/- regulatory T cells from peripheral blood lymphocytes of healthy *Mycobacterium tuberculosis*-infected humans. Microbes Infect 2007; **9**:1325–32.

88 Roberts T, Beyers N, Aguirre A, Walzl G. Immunosuppression during active tuberculosis is characterized by decreased interferon-gamma production and CD25 expression with elevated forkhead box P3, transforming growth factor- beta, and interleukin-4 mRNA levels. J Infect Dis 2007; **195**:870–8.

89 Davids M, Pooran AS, Pietersen E *et al*. Regulatory T cells subvert mycobacterial containment in patients failing extensively drug-resistant tuberculosis treatment. Am J Respir Crit Care Med 2018; **198**:104–16.

90 Riou C, Berkowitz N, Goliath R *et al*. Analysis of the Phenotype of Mycobacterium tuberculosis-specific $CD4^+$ T cells to discriminate latent from active tuberculosis in HIV-uninfected and HIV-infected individuals. Front Immunol 2017; **8**:968.

91 Adekambi T, Ibegbu CC, Cagle S *et al*. Biomarkers on patient T cells diagnose active tuberculosis and monitor treatment response. J Clin Invest 2015; **125**:1827–38.

92 Venigalla RK, Tretter T, Krienke S. Reduced $CD4^+CD25^-$ T cell sensitivity to the suppressive function of $CD4^+CD25^{high}CD127^{-/low}$ regulatory T cells in patients with active systemic lupus erythematosus. Arthritis Rheum 2008; **58**:2120–30.

93 Bhela S, Kempsell C, Manohar M *et al*. Nonapoptotic and extracellular activity of granzyme B mediates resistance to regulatory T cell (Treg) suppression by $HLA\text{-}DR^-CD25^{hi}CD127^{lo}$ Tregs in multiple sclerosis and in response to IL-6. J Immunol 2015; **194**:2180–18.

94 Kawamoto K, Pahuja A, Nettles A *et al*. Downregulation of TGF-βRII in T effector cells leads to increased resistance to TGF-β-mediated suppression of autoimmune responses in type I diabetes. Autoimmunity 2012; **45**:310–9.

95 Haufe S, Haug M, Schepp C *et al*. Impaired suppression of synovial fluid $CD4^+CD25^-$ T cells from patients with juvenile idiopathic arthritis by $CD4^+CD25^+$ Treg cells. Arthritis Rheum 2011; **63**:3153–62.

96 Day CL, Abrahams DA, Bunjun R *et al*. PD-1 Expression on *Mycobacterium tuberculosis*-specific CD4 T cells is associated with bacterial load in human tuberculosis. Front Immunol 2018; **9**:1995.

97 Amaral EP, Machado de Salles É, Barbosa Bomfim CC *et al*. Inhibiting adenosine receptor signaling promotes accumulation of effector $CD4^+$ T cells in the lung parenchyma during severe tuberculosis. J Infect Dis 2019; **219**:964–74.

98 Phillips BL, Mehra S, Ahsan MH *et al*. LAG3 expression in active *Mycobacterium tuberculosis* infections. Am J Pathol 2015; **185**:820–33.

99 Gotot J, Gottschalk C, Leopold S *et al*. Regulatory T cells use programmed death 1 ligands to directly suppress autoreactive B cells *in vivo*. Proc Natl Acad Sci USA 2012; **109**:10468–73.

100 Patterson SJ, Pesenacker AM, Wang AY *et al*. T regulatory cell chemokine production mediates pathogenic T cell attraction and suppression. J Clin Invest 2016; **126**:1039–351.

101 Barchet W, Price JD, Cella M *et al*. Complement-induced regulatory T cells suppress T-cell responses but allow for dendritic-cell maturation. Blood 2006; **107**:1497–504.

102 Kemper C, Chan AC, Green JM *et al*. Activation of human $CD4^+$ cells with CD3 and CD46 induces a T-regulatory cell 1 phenotype. Nature 2003; **421**:388–92.

103 Ikeda T, Hirata S, Fukushima S *et al*. Dual effects of TRAIL in suppression of autoimmunity: the inhibition of Th1 cells and the promotion of regulatory T cells. J Immunol 2010; **185**:5259–67.

104 Yi Z, Lin WW, Stunz LL, Bishop GA. The adaptor TRAF3 restrains the lineage determination of thymic regulatory T cells by modulating signaling via the receptor for IL-2. Nat Immunol 2014; **15**:866–74.

105 Truscott SM, Abate G, Price JD *et al*. CD46 engagement on human $CD4^+$ T cells produces T regulatory type 1-like regulation of antimycobacterial T cell responses. Infect Immun 2010; **78**:5295–306.

106 Bryce PJ, Oyoshi MK, Kawamoto S *et al*. TRAF1 regulates Th2 differentiation, allergic inflammation and nuclear localization of the Th2 transcription factor, NIP45. Int Immunol 2006; **18**:101–11.

107 Brunner T, Kasibhatla S, Pinkoski MJ *et al*. Expression of Fas ligand in activated T cells is regulated by c-Myc. J Biol Chem 2000; **275**:9767–72.

108 Zhang XR, Zhang LY, Devadas S *et al*. Reciprocal expression of TRAIL and CD95L in Th1 and Th2 cells: role of apoptosis in T helper subset differentiation. Cell Death Differ 2003; **10**:203–10.

109 Xie J, Wang H. Semaphorin 7A as a potential immune regulator and promising therapeutic target in rheumatoid arthritis. Arthritis Res Ther 2017; **19**:10.

110 Sun X, Yamada H, Shibata K *et al*. CD30 ligand/CD30 plays a critical role in Th17 differentiation in mice. J Immunol 2010; **185**:2222–30.

111 Khader SA, Bell GK, Pearl JE *et al*. IL-23 and IL-17 in the establishment of protective pulmonary CD4+ T cell responses after vaccination and during *Mycobacterium tuberculosis* challenge. Nat Immunol 2007; **8**:369–77.

112 Rosain J, Kong XF, Martinez-Barricarte R *et al*. Mendelian susceptibility to mycobacterial disease: 2014–2018 update. Immunol Cell Biol 2019; **97**:360–7.

113 Caretto D, Katzman SD, Villarino AV *et al*. Cutting edge: the Th1 response inhibits the generation of peripheral regulatory T cells. J Immunol 2010; **184**:30–4.

114 Diller ML, Kudchadkar RR, Delman KA *et al*. Balancing inflammation: the link between Th17 and regulatory T cells. Mediators Inflamm 2016; **2016**:6309219.

115 Ye ZJ, Zhou Q, Du RH *et al*. Imbalance of Th17 cells and regulatory T cells in tuberculous pleural effusion. Clin Vaccine Immunol 2011; **18**:1608–15.

116 Bhaumik S, Basu R. Cellular and molecular dynamics of Th17 differentiation and its developmental plasticity in the intestinal immune response. Front Immunol 2017; **8**:254.

117 Snook JP, Kim C, Williams MA. TCR signal strength controls the differentiation of $CD4^+$ effector and memory T cells. Sci Immunol 2018; **3**:eaas9103.

118 Baecher-Allan C, Viglietta V, Hafler DA. Inhibition of human $CD4^+CD25^{+/high}$ regulatory T cell function. J Immunol 2002; **169**:6210–7.

119 Moguche AO, Musvosvi M, Penn-Nicholson A *et al*. Antigen availability shapes T cell differentiation and function during tuberculosis. Cell Host Microbe 2017; **21**:695–706.

120 Sakai S, Kauffman KD, Sallin MA *et al*. CD4 T Cell-derived IFN-γ plays a minimal role in control of pulmonary *Mycobacterium tuberculosis* infection and must be actively repressed by PD-1 to prevent lethal disease. PLOS Pathog 2016; **12**:e1005667.

121 Lyadova IV, Tsiganov EN, Kapina MA *et al*. In mice, tuberculosis progression is associated with intensive inflammatory response and the accumulation of Gr-1 cells in the lungs. PLOS ONE 2010; **5**:e10469.

122 Orme IM, Robinson RT, Cooper AM. The balance between protective and pathogenic immune responses in the TB-infected lung. Nat Immunol 2015; **16**:57–63.

123 Gideon HP, Phuah J, Myers AJ *et al*. Variability in tuberculosis granuloma T cell responses exists, but a balance of pro- and anti-inflammatory cytokines is associated with sterilization. PLOS Pathog 2015; **11**:e1004603.

124 Fletcher HA, Snowden MA, Landry B *et al*. T-cell activation is an immune correlate of risk in BCG vaccinated infants. Nat Commun 2016; **7**:11290.

125 Pandiyan P, Zheng L, Ishihara S *et al*. $CD4^+CD25^+$ $Foxp3^+$ regulatory T cells induce cytokine deprivation-mediated apoptosis of effector $CD4^+$ T cells. Nat Immunol 2007; **8**: 1353–62.

126 Bettini M, Vignali DA. Regulatory T cells and inhibitory cytokines in autoimmunity. Curr Opin Immunol 2009; **21**:612–8.

127 Vignali DA, Collison LW, Workman CJ. How regulatory T cells work. Nat Rev Immunol 2008; **8**:523–32.

128 Gondek DC, Lu LF, Quezada SA *et al*. Cutting edge: contact-mediated suppression by $CD4^+CD25^+$ regulatory cells involves a granzyme B-dependent, perforin-independent mechanism. J Immunol 2005; **174**:1783–6.

129 Onishi Y, Fehervari Z, Yamaguchi T, Sakaguchi S. Foxp3+ natural regulatory T cells preferentially form aggregates on dendritic cells *in vitro* and actively inhibit their maturation. Proc Natl Acad Sci USA 2008; **105**:10113–8.

130 Fallarino F, Grohmann U, Hwang KW *et al*. Modulation of tryptophan catabolism by regulatory T cells. Nat Immunol 2003; **4**:1206–12.

131 Huang CT, Workman CJ, Flies D *et al*. Role of LAG-3 in regulatory T cells. Immunity 2004; **21**:503–13.

132 Deaglio S, Dwyer KM, Gao W *et al*. Adenosine generation catalyzed by CD39 and CD73 expressed on regulatory T cells mediates immune suppression. J Exp Med 2007; **204**: 1257–65.

133 Bopp T, Becker C, Klein M *et al*. Cyclic adenosine monophosphate is a key component of regulatory T cell-mediated suppression. J Exp Med 2007; **204**:1303–10.