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Review

The immune exhaustion paradox: activated functionality during chronic bacterial infections

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Abstract

Co-inhibitory molecules, such as cytotoxic T-lymphocyte-associated protein 4 (CTLA-4) and programmed cell death protein 1 (PD-1), known as immune checkpoints, regulate the activity of T and myeloid cells during chronic viral infections and are well-established for their roles in cancer therapy. However, their involvement in chronic bacterial infections, particularly those caused by pathogens endemic to developing countries, such as *Mycobacterium tuberculosis* (*Mtb*), remains incompletely understood.

Cytokine microenvironment determines the expression of co-inhibitory molecules in tuberculosis: Results indicate that the cytokine IL-12, in the presence of *Mtb* antigens, can enhance the expression of co-inhibitory molecules while preserving the effector and memory phenotypes of CD4+ T cells.

Intersection of immune checkpoint inhibitors' role in cancer therapy and active tuberculosis: As discussed, co-inhibitory molecules' expression is crucial for effectively controlling inflammation during chronic bacterial infections. It has been suggested that monoclonal antibodies (mAbs) developed for cancer immunotherapy, known as immune checkpoint inhibitors (ICIs), may be associated with the reactivation of latent tuberculosis (LTB), though this occurrence has been rarely reported.

Immune checkpoint molecules function as a "brake" to protect the host from the pathological effects of the immune response during chronic bacterial infections, which contrasts with the concept of exhaustion in the context of cancer. This means cells expressing co-inhibitory molecules on their surface can be paradoxically activated, as suggested in this review.

Key words: exhaustion; PD-1; PD-L1; CTLA-4; intracellular.

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Introduction

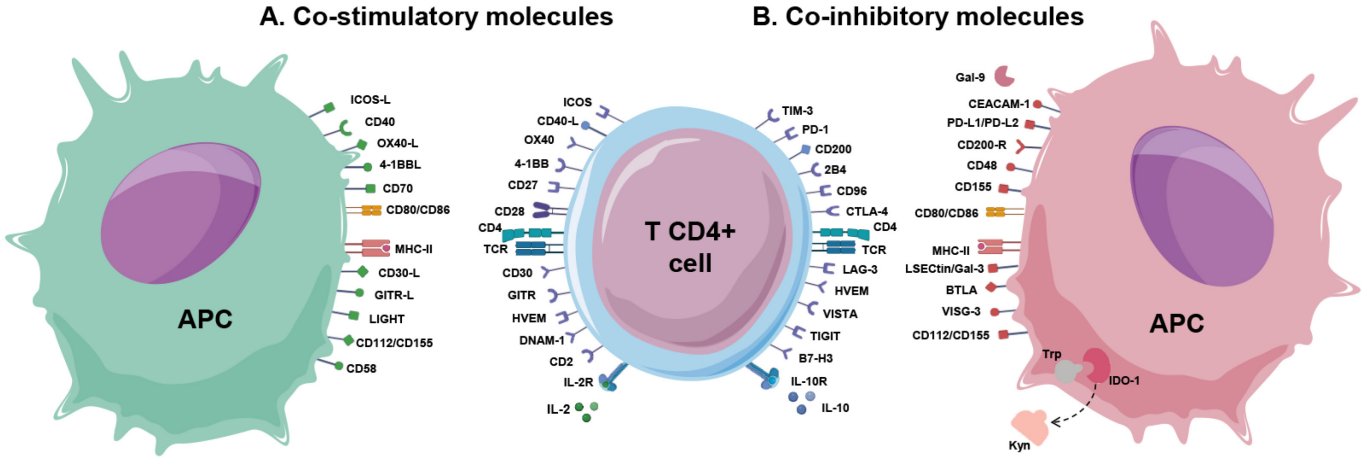
The immune system can react against infection while maintaining homeostasis through a balanced negative regulation. The activation of T cells is a process that involves three signals: 1) engagement of the T cell receptor (TCR) with the pathogen peptide presented by the major histocompatibility complex molecules (MHC) in the antigen-presenting cells (APC), 2) co-stimulation signals, and 3) cytokines microenvironment. Thus, these co-stimulatory molecules should coordinate with the co-inhibitory molecules to modulate the inflammatory response. As the number of co-inhibitory molecules (Figure 1) continues to grow, the need to establish their role in disease becomes more evident.

Brunet *et al.* first described co-inhibitory molecules in 1987 and they studied the CTLA-4 molecule [1]. The function of this molecule was not elucidated until Krummel and Allison found that this molecule acts as a "brake" on T cell activation by binding co-stimulatory molecules CD80 and CD86 present in APC [2]. Ishida *et al.* in 1999 described PD-1 and demonstrated that it is a negative regulator of T-cell responses by interacting with its ligand, PD-L1, down-modulating the TCR signaling, thus interfering with the activation of the T cells (Figure 2) [3]. Iwai *et al*. also showed that PD-1 and PD-L1 blockade by monoclonal antibodies (mAbs) is a promising strategy to fight against cancer in animal experiments [4]. This finding paved the way for using PD-1 as a therapeutic target in cancer patients' immunotherapy, and it stimulated worldwide intensive research in clinical trials to treat different forms of cancer by disrupting T-cell tolerance to cancer cell antigens.

The success of the monoclonal antibodies that block the co-inhibitory molecules, better known as checkpoint inhibitors in cancer therapy, encouraged investigation of the role of these molecules in chronic infections.

This review focuses on the chronic infections caused by intracellular bacteria that are endemic in developing countries, including *Mycobacterium*

Figure 1. Co-stimulatory and co-inhibitory signals in the T cell and antigen-presenting cells (APCs).



A: The co-stimulatory molecules in T cells, when engaged with their ligands on APCs provide positive signals that contribute to T-cell proliferation and effector functions. **B:** Co-inhibitory molecules in T cells act as checkpoints when they interact with their ligands, on APC. This contributes to fine-tuning immune activation, preventing excessive inflammatory responses while maintaining immune homeostasis.

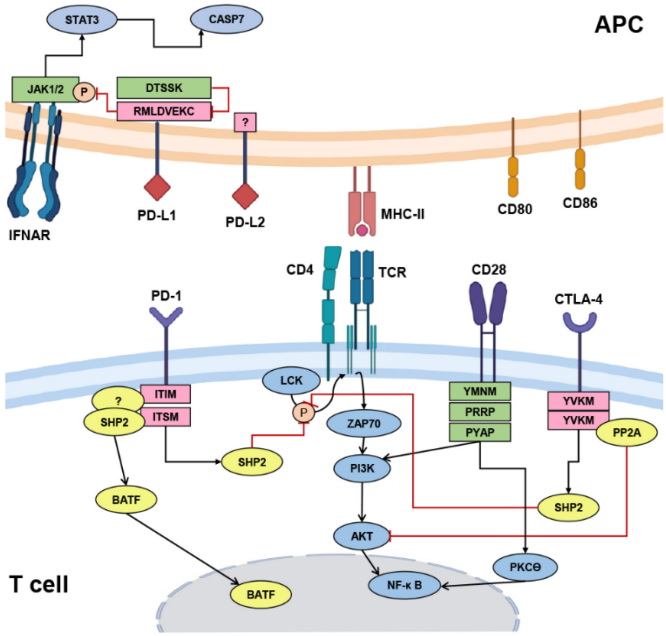
tuberculosis (*Mtb*), *M. leprae*, *Burkholderia* spp., *Brucella* spp., *Borrelia* spp., *Salmonella* spp., and *Chlamydia* spp., and presents updated information on the described role of co-inhibitory molecules expressed in T and myeloid cells, and the use of immune checkpoint inhibitors in the clinical setting.

Immune response to intracellular bacterial pathogens

Pathogenic bacteria actively seek out an environment inside the cells where they can persist despite the harsh conditions created by the immune system. The interactions between host cells and the pathogen are regulated by 1) the elicited cytokine response as the result of the interaction of host cells' pattern recognition receptors (PRR) and bacterial pathogen-associated molecular patterns (PAMP) and 2) the activation of T cells after the engagement of the TCR with the MHC in APC, and T cell co-receptors CD4 and CD8, as well as co-stimulatory molecules CD28 and CD80/86 [5].

Once the bacteria have entered the host, to prevent intracellular infection, immune cells must discriminate between commensal and pathogenic bacteria via PRR,

Figure 2. Activation and inhibition pathways in CD4+ T cells and antigen-presenting cells.



The T cell activation signaling pathways are triggered by the T cell receptor (TCR) interaction with the peptide-major histocompatibility complex II (MHC-II) from the antigen-presenting cell (APC), and CD28 interacting with CD80 and/or CD86; this activation can be countered by programmed cell death protein 1 (PD-1) and the cytotoxic T-lymphocyte-associated protein 4 (CTLA-4) signalization. CTLA-4 can bind CD80 or CD86, recruiting the phosphatases src homology 2 (SH2) domain-containing tyrosine phosphatase-2 (SHP2) and the protein phosphatase 2A (PP2A) via the YVKM motif in its cytoplasmic domain. SHP2 recruitment results in attenuation of TCR signaling by dephosphorylating the CD3ζ chain. PP2A recruitment results in downstream dephosphorylation of protein kinase B (AKT). PD-1 ligation by PD-L1 or PD-L2 also recruits SHP2 to the immunoreceptor tyrosine switching motif (ITSM) domain, resulting in decreased activation of transcription factors, such as nuclear factor-κB (NF-κB), which are important for driving T cell activation, proliferation, effector functions, and survival. In addition, PD-1 can inhibit T cell functions by increasing the expression of transcription factors such as the basic leucine zipper ATF-like transcription factor (BATF), which can further counter effector transcriptional programs. In the APC, the ligation of PD-L1 can counteract interferon-mediated apoptosis, with its motif RMLDVEKC inhibiting signal transducer and activator of transcription 3 (STAT3) phosphorylation. DTSSK motif has been shown to inhibit their contiguous motif and act as a negative regulator.

which are present in the plasma membrane or within intracellular compartments. The main PRR described in this context is the toll-like receptor (TLR) family, located in the plasma membrane and endosomal compartments.

The recognition of PAMPs via TLR leads to the oligomerization of the myeloid differentiation primary response 88 (MyD88) adaptor protein, consequently activating mitogen-activated protein kinase (MAPK) and transcription factor nuclear factor-kappa B (NF-κB) signaling. This signaling pathway leads to the production of the pro-inflammatory cytokines IL-1, IL-2, IL-6, IL-8, IL-12; and tumor necrosis factor-α (TNFα); and the production of chemokines, like monocyte chemoattractant protein-1 (MCP-1), macrophage inflammatory protein-2 (MIP-2), and the interferon gamma-induced protein 10 (CXCL10). The induction of TLR signaling also contributes to the polarization of macrophages to their pro-inflammatory phenotype and the upregulation of MHC, and co-stimulatory molecules [6]. Other PRRs can recognize intracellular bacteria, like the NOD-like receptors, AIM2-like receptors, and STING/cGAS sensors that are in the cytoplasm, and the C-type lectins and scavenger receptors located in the plasma membrane of the cell [7]. These receptors can activate innate immunity and stimulate the production of cytokines that act locally and systemically as protective mediators that function as the bridge to set the immune adaptive response, generating antigen-specific cells.

The main characteristic of intracellular bacteria is the ability to make the host cells internalize them and evade their immune sensing mechanisms. Examples of bacterial modulation of the host cells are a) the modulation of bacterial antigens, which interferes with pathogen recognition, b) inhibition of phagocytosis, c) polarization of cells to an anti-inflammatory phenotype, d) microbial secretion systems that allow the bacteria to enter the host cells, and e) modulation of cell surface receptors, like the MHC and PD-L1 [8].

There is a lack of information to help us understand the mechanisms and pathways of host-intracellular bacteria interaction that may lead to new therapeutic targets beyond antibiotic therapy to control the increased list of pathogenic intracellular bacteria; so, the role of these co-inhibitory molecules deserves further investigation. Table 1 summarizes the bacteria studied in the context of co-inhibitory mechanisms.

Co-inhibitory molecules in tuberculosis scenarios

We focused on tuberculosis (TB) because it is the most extensively studied intracellular chronic bacterial infection regarding co-inhibitory molecules. However, findings regarding other bacteria are also mentioned in this review. The chronic state of this infection is characterized by the presence of granulomas, which serve as a niche where bacteria are contained [9]; crosstalk exits between the immune cells from the myeloid core and the lymphocytic cuff with the cells from the vascular compartment in the surrounding tissue orchestrated by cytokines [10]. It is important to mention that *Mtb* infection has been modeled in vivo, in vitro, and in silico, to mimic the host immune response; nevertheless, a lot of questions remain regarding co-inhibitory molecules´ expression in granuloma cells and whether it is an effective or deleterious mechanism.

Table 1. Diseases caused by intracellular bacteria.

Facultative intracellular bacteria		
Granulomatous infection	**Pathogen**	**Target cells**
Tuberculosis	*Mycobacterium tuberculosis*	Macrophages Dendritic cells
Leprosy	*Mycobacterium leprae*	Macrophages Schwann cells
Typhoid fever	*Salmonella spp.*	Epithelial cells M cells Macrophages Dendritic cells Polymorphonuclear neutrophils
Brucellosis	*Brucella spp.*	Macrophages
Melioidosis	*Burkholderia spp.*	Epithelial cells Macrophages
Non-granulomatous infection	Pathogen	Target cells
Lyme disease	*Borrelia spp.*	Macrophages Dendritic cells
Obligate intracellular bacteria		
Infection	**Pathogen**	**Target cells**
Chronic pneumonia	*Chlamydia pneumoniae*	Epithelial cells
Trachoma	*Chlamydia trachomatis*	Epithelial cells

Cytokine microenvironment determines the expression of co-inhibitory molecules in tuberculosis

The hallmark response during *Mtb* infection is the production of IL-12 by infected macrophages, which stimulates the differentiation of T cells to a pro-inflammatory T-helper (Th) 1 phenotype that produces cytokines such as IFN-γ, TNF-α, and IL-2. Recently, the evidence demonstrated that the IFN-γ-mediated response inhibited inflammation during TB [11]. This fact led us to the idea that co-inhibitor mechanisms might be elicited that can modulate the pro- and anti-inflammatory signals.

The first time CTLA-4 was reported in the context of chronic bacterial disease was in TB patients' peripheral blood mononuclear cells (PBMC) after in vitro stimulation with *Mtb* antigens [12], meaning that the expression of CTLA-4 was related to an immunomodulatory checkpoint, rather than a sign of terminal exhaustion, unlike chronic viral infections, such as human immunodeficiency virus (HIV) or the experimental lymphocytic choriomeningitis virus (LCMV) [13,14]. This notion was further demonstrated because treated TB patients had increased levels of CTLA-4 expression on T CD4+ cells [15,16]; whereas, in healthy tuberculin reactors, the CTLA-4 expression level was lower than in TB patients [12,15,16].

In in vitro experiments, the IL-12 released by in vitro *Mtb*-infected macrophages increased IFN-γ production and CTLA-4 expression in T cells [12]. In contrast, the immunoregulatory cytokine IL-10 inhibited mycobacterial growth in human monocytes and promoted CTLA-4 and IFN-γ expression [12]. This finding leads us to the idea that T cells activated and differentiated to a pro-inflammatory phenotype can still produce IFN-γ while expressing CTLA-4 on their surface. However, the direct effect of CTLA-4 in clearing the infection has not been demonstrated.

Likewise, PD-1 was minimally expressed in PBMCs from active TB patients [17,18] predominating in CD4+ T cells [15,16]. Elevated levels of PD-1 expression were also described in pleural fluid mononuclear cells (PFMC) and bronchoalveolar lavage (BAL), mainly the effector memory subtype CD4+ [19,20] and activated T cells [17,19,21,22]. After in vitro stimulation with *Mtb* antigens, PD-1 positive (PD-1+) T cells increased IL-12R expression and enhanced proliferation [20].

These results show that the cytokine IL-12, in the presence of *Mtb* antigens, can promote the expression of co-inhibitory molecules while maintaining the effector and memory phenotype on CD4+ T cells. Also, cytokine IL-10 can downregulate the expression of these molecules, in addition to undergoing a loss of effector functions and mycobacterial control. Nevertheless, much more work beyond the in vitro setting is required to comprehend cytokine dynamics and signaling pathways that trigger the expression of these markers.

Cytotoxicity is modulated by the expression of co-inhibitory molecules

Cytotoxic cells control intracellular infections by killing compromised host cells as part of the adaptive immune response by releasing effector molecules, and increasing local inflammation. A proposed mechanism to control the extent of the consequent cytopathic damage is the expression of co-inhibitory molecules on the cytotoxic cell population.

It was shown in an animal model that PD-1+ CD8+ T cells increase over time on newly primed cells. Then, PD-1 expression declined throughout the beginning of the chronic infection [23]. Peripheric CD8+ T lymphocytes from patients with active TB showed increased PD-1 expression and a higher IFN-γ concentration [22]; also, after in vitro stimulation with *Mtb* antigens, PBMC from household healthy contacts of patients with active TB had higher proportions of PD-1+ CD8+ T cells [24].

Nonetheless, after stimulating PFMC with *Mtb* antigens and blocking the PD-1/PD-L1 axis, the degranulating cytotoxic lymphocytes increased in vitro [25]. This finding was also seen in an animal model, where higher numbers of granzyme B-positive and perforin-positive cells were found in the *Mtb*-infected lungs of PD-1−/− and PD-L1−/− mice than in wild-type mice [26]. Lower cytotoxicity in T CD8+ PD-1+ from active TB patients can be attributed to the basic leucine zipper ATF-like transcription factor (BATF) (Figure 2). Its knockdown increases IL-2, IFN-γ production, and T-cell proliferation [27]. We hypothesize that the CD8+ T cells from infected tissue are downmodulated locally by PD-1/PD-L1; while peripherally, these cells are still active while expressing PD-1 in the absence of their ligand, PD-L1.

Natural killer (NK) cells, the cytotoxic cell population that belongs to the innate immunity, have also been explored in the context of *Mtb* infection, observing in PFMC from patients with active TB an increase in the secretion of IFN-γ with a significant augment of PD-1 on their surface [28], but their lytic degranulation was restored only after blocking the PD-1/PD-L1 axis, and even IFN-γ production was higher [17].

This fact leads us to the idea that the cytotoxic degranulation from both CD8+ T cells and NK cells is restrained when the marker PD-1 is expressed to control the direct cytopathic effect in the tissue surrounding the infected area, in this case, the granuloma. Rather, the production of IFN-γ is decreased during PD-1 expression but not absent, indicating that the cell maintains its effector capacity while expressing co-inhibitory molecules.

Regulatory T cells balance inflammation by engaging co-inhibitory molecules

There is a recurring debate as to whether regulatory T cells (Treg) are beneficial or detrimental in *Mtb* infection due to their anti-inflammatory function, which can counterbalance the inflammatory response in the lungs by producing IL-10 and by promoting the apoptosis of Th1-type cells [29].

Recently, a study described the presence of a Treg infiltrate in the myeloid core of human granulomas that expressed PD-1 and kept proliferating even more than the lymphocytes in the cuff [10]. Nevertheless, the function of this co-inhibitory molecule was not described. The accumulation of Treg in the lung is explained by the modulation induced by *Mtb* antigens that modulate antigen-presenting cells (APC), leading to the secretion of chemokines, such as CCL22 and CCL17, which attract Treg to the infection site. Furthermore, the interaction of PD-L1 expressed on the surface of this infected APC with the PD-1 on Treg can cause this regulatory population to expand [29,30]. Also, significantly higher levels of PD-1 in Tregs from PBMC among TB patients have been described. The PD-1 in vitro blockade of these cells decreased the Treg antigen-specific suppressive activity [29].

These results demonstrate that the co-inhibitory axis PD-1/PD-L1 not only has a role in the control of the cytotoxic activity in CD8+ cells but also in promoting the proliferation and functionality of the Treg recruited to promote a balance between anti-inflammatory and pro-inflammatory states.

Co-expression of co-inhibitory molecules and immune exhaustion

It is well known that chronic viral infections lead to a state of exhaustion related to the expression of co-inhibitory molecules [14]. *Mtb* infections produce mainly active CD4+ T cells that express CTLA-4 or PD-1, but there are few reports on the co-expression of these markers.

During the chronic stage of *Mtb* lung infection in mice, the co-expression of PD-1, CTLA-4, and the T cell immunoglobulin and mucin-containing domain 3 (TIM-3) has been reported, revealing the loss of IFN-γ, TNF, and IL-2 production [31]. This finding can be associated with the exhausted phenotype reported in chronic viral infections, where more than two co-inhibitor molecules simultaneously expressed in the T cells affect their function.

Moreover, there is evidence of immune exhaustion in HIV patients co-infected with *Mtb*, with an overall decreased proliferative response and Th1-type cytokines secretion. In contrast, in PBMC from patients with active TB disease who are non-HIV infected, there is low co-expression of the co-inhibitor molecules, CTLA-4, PD-1, and B- and the T-lymphocyte attenuator (BTLA) [32]. T cells in the lymphocyte cuff of the granuloma from *Mtb*-infected animals continue proliferating and producing IL-2, TNF, and IFN-γ while expressing PD-1 and LAG-3, indicating functionality [16]. This exhaustion state can be attributed to a chronic viral infection, not active TB. However, additional investigation is required to ascertain whether additional co-inhibitory markers and the cells' functional status are expressed simultaneously.

It is important to mention that when the PD-1/PD-L1 co-inhibitory axis is blocked with mAbs, other co-inhibitory markers can be upregulated, like CTLA-4 or lymphocyte-activation gene 3 (LAG-3) [33], which can compensate for the absence of a modulatory signaling pathway, attributed to the biological redundancy of co-inhibitory molecules.

This finding implies that during TB infection, co-inhibitory molecules can function as a brake to stop an exacerbated immune response, and the expression of more than three of these molecules on the surface of the T cells may be needed to acquire the immune exhaustion state, hindering effector T cells. However, extensive research is needed to demonstrate the expression patterns of multiple co-inhibitory molecules during *Mtb* chronic infection.

Co-inhibitory molecules are crucial to control infection and establish memory populations

As suggested in the present review, the expression of co-inhibitory molecules has a major role in controlling exacerbated inflammatory responses during chronic intracellular bacterial infections.

In *Mtb*-infected animal models, the absence of the PD-1/PD-L1 axis resulted in uncontrolled bacterial growth, large and immature granulomas, deformed and perforated lungs, neutrophil infiltration, lower T CD4+ cells in the lung [34,35], lower CD8+ T cell cytotoxic response , and elevated pro-inflammatory cytokines

like IL-1β, IL-6, and kynurenine in sera; but IFN-γ and IL-12 levels decreased in the lungs, correlating with reduced survival rates [33]. This finding proves that co-inhibitory molecules have a major role in controlling the pathological effects of inflammation and maintaining bacterial restraint.

Once the bacterial stimuli decrease due to effective infection control with anti-TB therapy in patients and animal models, the level of active antigen-specific CD4+ T cells and the expression of co-inhibitory molecules decline [18,36,37], proving that the expression of these molecules can be reversible once the pro-inflammatory state has ended.

In addition, during active and chronic *Mtb* infection, the memory CD4+ T cells subset expressed PD-1 [20,38]. Interestingly, after the bacille Calmette-Guérin (BCG) vaccination in healthy adults, the PD-1+ fraction of CD8+ T cells from PBMC displayed enhanced proliferative capacity after BCG stimulation in vitro, compared to the total CD8+ population [15].

All these findings support the idea that PD-1 is related to effectively controlling bacterial infection while maintaining tissue homeostasis and suggest that their expression on memory populations could be related to a control mechanism for rapid activation after the engagement with the specific bacterial antigen.

Immunoregulatory function of PD-L1, PD-L2, and IDO-1 on myeloid cells in *Mtb* infection

The control of *Mtb* is primarily dependent on bactericidal mechanisms triggered by activated infected M1 macrophages. However, other myeloid cells are present in the granuloma microenvironment, like neutrophils, M2 macrophages, foamy macrophages, and mast cells; and all of these can express the ligands for the co-inhibitory molecules in T cells in response of pro-inflammatory chronic stimuli, like PD-L1 [10].

Monocytes obtained from PBMCs of patients with pulmonary TB expressed high levels of PD-L1 and PD-L2 [18,22,39] and even higher levels in cells obtained from PFMC [21]. In addition, the dendritic cells (DC), classical macrophages, and foamy macrophages in the granuloma from animal models, showed increased expression of PD-L1 at later stages of infection [40,41]. Classical neutrophils and low-density granulocytes, similar to the phenotype of the myeloid-derived suppressor cells (MDSC) described in the cancer context, also express high levels of PD-L1 [42,43].

A very noticeable characteristic of the myeloid-rich regions of the *Mtb* granuloma is the co-expression of PD-L1 and indoleamine- 2,3- dioxygenase (IDO-1), mainly in macrophages, multinucleated giant cells, and neutrophils [10]. The enzyme IDO-1 depletes L-tryptophan in the cells, resulting in cell cycle inhibition [44]. The accumulation of its metabolite kynurenine induces apoptosis of T cells, as well as promotes the differentiation to Treg subtype in the core infiltrating cells that produce TGF-β [10], generating an immunoregulatory niche in the myeloid core of the granuloma [30]. This enzyme can also deprive the intracellular bacteria of tryptophan by metabolizing this essential amino acid [45]. The upregulation of IDO-1 was observed in vitro in macrophage infection using the strain H37Rv, but not when infecting with the non-virulent strains BCG or H37Ra; thus, proven to be a strain-dependent mechanism, and an indicator that may be of clinical use in the study of TB disease [46].

Another interesting feature of TB granulomas is that PD-L1+ cells are present in higher numbers compared to PD-1+ cells [10,33], meaning that the myeloid core serves as an immunoregulatory region.

Together, these findings suggest that in response to pathogenic mycobacteria, the cells can modulate the granuloma, creating an immunosuppressive niche by creating enough PD-1 ligands to maintain the co-inhibitory axis. However, further investigation is needed on the intrinsic function of PD-L1 of myeloid cells to conclude if this axis is beneficial or detrimental during chronic infection.

Intersection of immune checkpoint inhibitors' role in cancer therapy and active tuberculosis

As presented, the blockade of a co-inhibitory axis can be unfavorable in effectively controlling mycobacteria and maintaining homeostasis during chronic infection. The mAbs designed for cancer immunotherapy, known as immune checkpoint inhibitors (ICI), can be related to the reactivation of latent TB (LTB), but this has been minimally reported [47–54] (Table 2). An in vitro three-dimensional model was recently reported, where the use of an anti-PD-1 mAb resulted in increased *Mtb* growth and elevated TNF-α, suggesting that an excess of this cytokine can be a primary driver of increased TB exacerbations in cancer patients receiving ICI therapy [55].

It is important to reach a worldwide consensus regarding intensive research for LTB in cancer patients and establish guidelines for the correct management of candidates for ICI therapy before starting its use. Also, the suggestion that complications are related to increased pro-inflammatory cytokines can indicate that specific inhibitors may be needed concomitantly with ICI. Other factors like the location of the malignancy, high TB prevalence in endemic regions, the presence of

an immunosuppression state, or other comorbidities may also be considered.

Role of co-inhibitory signals in granulomatous infections caused by intracellular bacteria

Granulomatous inflammation, a morphologic characteristic of chronic inflammation, can be produced by bacterial pathogens other than *Mtb*. Nevertheless, comparable results concerning co-inhibitory molecules have been documented.

Leprosy is still common in developing nations, where it has a substantial negative impact on people's health and well-being. These infections, caused by *Mycobacterium leprae*, manifest in two polar forms: lepromatous leprosy (LL) and tuberculoid leprosy (TL).

LL is characterized by an insufficient cellular immune response with bacteria spreading throughout the body, affecting multiple organs. *M. leprae* replicates within macrophages, forming granulomas known as lepromas, with a Th2-type immune response with high levels of IL-4 and the immunoregulatory cytokine IL-10. This Th2 bias impairs macrophage activation and the effective killing of intracellular bacteria, which explains the immune failure in patients. TL, on the other hand, is distinguished by a robust cellular immune response, with a Th1-type immune response and elevated IFN-γ levels. Th1 cells activate macrophages, making them more capable of killing intracellular bacteria [56].

In LL, IL-10 involvement inhibits antigen-specific T-cell proliferation and Th1 cytokine production [57]. The increase of these cytokines is paralleled by increased expression of IL-10 and CTLA-4, suggesting that the expression of this molecule serves as a mechanism to suppress excessive effector T cell responses and T cell proliferation [58]. PD-1 expression was found primarily on activated T cells from LL patients, and PD-L1 expression was significantly higher in APC [59].

LL patients have circulating monocytes with low expression of CD86 and CD28 on T-cells, consistent with an immune suppressive state [60]. It was proposed that CTLA-4 expressing cells remove CD80/CD86 mediated signaling by binding its ligands from APC, which results in impaired co-stimulation via CD28 and TCR signaling [61].

These findings lead us to propose that, in addition to looking into co-inhibitory markers, research should also look into co-stimulatory molecule expression, TCR and MHC expression, T cell phenotype, and cytokine profile to understand the dynamics of granulomatous infection.

Burkholderia spp. is a facultative intracellular opportunistic pathogen that causes melioidosis, a disease that can range from pneumonia to visceral abscesses, and in its chronic form, induces granulomatous lesions in multiple organs [62,63]. Interestingly, the reports regarding this bacterium point out that the expression of co-inhibitory molecules on immune cells can be strain-dependent as CD4+ T cells

Table 2. Reports of tuberculosis in cancer patients during immune checkpoint inhibitors therapy.

Clinical study	Cancer patients	Previous report of TB	Checkpoint inhibitors therapy	TB infection outcome	Ref
Case report	Hodkin´s lymphoma	No	Pembrolizumab (anti-PD-1)	Resolution of pulmonary tuberculosis.	[47]
Case report and review	Melanoma (2 cases)	No	Case1: Ipilimumab (anti-CTLA-4) Case 2: Atezolizumab (PD-L1)	Case 1: Death related to pulmonary TB. Case 2: Resolved infection	[48]
Case report	Case 1: Nasopharyngeal carcinoma; Case 2: Merkel lymphoma	No	Case 1: Nivolumab (anti-PD-1) Case 2: Pembrolizumab (anti-PD-1)	Case 1: Death related to pulmonary TB. Case 2: Resolution of pulmonary tuberculosis	[49]
Retrospective study	Lung cancer (76.9%)	Yes (LTB 18% of total reports)	Anti-PD-1 or anti-PD-L1 (1.3%)	12 cases of resolution of pulmonary tuberculosis cases, 1 case of death related to pulmonary TB.	[50]
Retrospective study	Non-small cell lung cancer (95%)	Yes (1%)	Anti-PD-1 (100%)	n/a	[51]
Retrospective study	Non-small cell lung cancer (100%)	Yes (ATB 84.6%, LTB 15.6%)	Anti-PD-1 (100%)	n/a	[52]
Retrospective study*	Lung cancer (77.7%)	n/a	Anti-PD-1 (100%)	Fair to good response to anti-TB treatment (71.4%) Poor response to anti-TB treatment (14.3%) Death related to TB (14.3%)	[53]
Case report and retrospective study	Lung cancer (56.5%) Melanoma (21.7%) Other (21.8%)	LTB (8.7%)	Anti-PD-1 (95.7%) Anti-PD-1 + anti-CTLA-4 (4.3%)	2 cases related directly related to pulmonary TB (8.7%)	[54]

ATB: active tuberculosis; LTB: latent tuberculosis; n/a: not-applicable; TB: tuberculosis. *NTM: non-tuberculous mycobacteria.

showed higher PD-1 levels when infected with the colony morphotype OS of *B. pseudomallei* [64].

Additionally, when PBMCs were infected with various strains of *Burkholderia* spp., only the heat-inactivated *B. pseudomallei* strain derived from a clinical spleen isolate (HI *B. pseudomallei* THE) was found to promote PD-1 expression. In contrast, the *B. pseudomallei* K9 strain and *B. thailandensis* did not induce this effect [65]. PD-L1 expression is increased in neutrophils infected in vitro with *B. pseudomallei*, and blockade of this ligand can restore CD4+ T cell proliferation and IFN-γ production [66]. These results suggest that co-inhibitory molecule expression can be strain-dependent; however, it is important to evaluate if this is a direct effect of the interaction of bacteria within the cells or if it is the result of the immunomodulatory mechanism of the cell to prevent inflammatory pathology.

Brucella species is the etiologic agent for brucellosis, a zoonotic disease, and its chronic form is also characterized by granulomatous inflammation. The expression of PD-1 on CD4+ and CD8+ T cells in PBMC from patients during the acute phase of infection with *B. melitensis* was higher than in the chronic brucellosis or convalescent groups [67]; only the CD8+ T cells PD-1+ LAG-3+ have limited function during chronic infection [68]. These results support our idea that the expression of these co-inhibitory molecules indicates cell activation and not an exhaustion state, as these molecules´ role is to prevent the extent of inflammation, which agrees with the findings regarding *Mtb*.

The outer membrane protein Omp25 of *Brucella* spp. negatively regulates the signaling pathways of TLR4 that produce IL-12 at both transcriptional and posttranscriptional levels and, in an Omp25 protein-dependent manner, PD-1 is induced, adding to the knowledge of the importance of considering the direct role of bacterial strains or their components in the stimulation of the expression of co-inhibitory molecules [69].

Salmonella spp. causes self-limiting gastroenteritis to life-threatening typhoid fever in humans, or, in rare cases, promotes a granulomatous inflammatory response [70]. *Salmonella* spp. pathogenicity island 2, which belongs to T3SS, is largely responsible for the induction of PD-1 in CD4+ T cells [71]; the synergistic expression of PD-L1 and IFN-γ has also been reported on infected intestinal epithelial cell lines [72]. Also, during the early and late phases of a chronic *Salmonella* spp. infection, APC express co-stimulatory (CD40, CD80, and CD86) and co-inhibitory molecules (PD-L1 and PD-L2) simultaneously [73]. In contrast, antigen-specific CD8+ T cells express PD-1 continuously during the late stages of infection. By blocking this axis, primary infected APCs are eliminated, and CD8 T cell proliferation is restored [73]. This reasserts the findings with *Mtb*, where the CD4+ cells remain active, and only the CD8+ T cells are deprived of their cytotoxic activity.

Considerable research must still be conducted to determine which bacterial factors and signaling pathways are involved in expressing co-inhibitory markers in the mentioned pathogens.

Co-inhibitory signals in persistent infections by intracellular facultative bacteria

Intracellular facultative bacteria, such as *Borrelia* spp., are responsible for chronic, non-granulomatous infections, such as Lyme disease, even though research on the role of co-inhibitory molecules in their respective context is lacking.

In a mouse infection model with *Borrelia burgdorferi*, PD-1, and PD-L1 were significantly upregulated on CD4+ T cells and antigen-presenting cell subsets, respectively. The PD-1/PD-L1 pathway does not impact bacterial clearance. Still, it does impact T cell expansion and accumulation in the ankle joint and popliteal lymph nodes, suggesting that this pathway may shape the T cell populations in affected tissues [74].

Co-inhibitory signals in chronic infections by obligate intracellular bacteria

The obligate intracellular bacterium *Chlamydia pneumoniae* can cause bronchitis, pneumonia, and other respiratory infections. In certain cases, it can also result in chronic lung disease. *C. pneumoniae* infects respiratory epithelial cells through endocytosis, creating an inclusion, an intracellular niche where it can multiply [75].

Elevated expression of PD-1 and PD-L1 was observed in lung tissue in a murine model of intranasal infection of *Chlamydia muridarum*. PD-1 was expressed on CD8+ and CD4+ T cells, and PD-L1 was increased in neutrophils. In addition, myeloid and plasmacytoid DC had increased expression of PD-L1 and PD-L2. The inhibition of PD-1 and PD-L1 during acute infection can return transpulmonary resistance and IFN-γ to baseline levels [75].

Additional immunoregulatory molecules, such as ICOS and its ligand ICOS-L, are known to function as co-stimulatory molecules. The interaction of these

molecules is required for CD4+ T cells to inhibit chlamydial growth in cultured lung fibroblasts [76].

Chlamydia trachomatis can cause genital infections, including urethritis in men; and urethritis, cervicitis, and pelvic inflammatory disease in women; by entering host cells through endocytosis into the epithelial cells of the genital tract, including the urethra, cervix, and endometrium. In addition, when infecting the eye conjunctiva, it produces irritation and inflammation, known as trachoma. Since trachoma is a neglected tropical disease, programs to improve sanitation, promote facial hygiene, and develop tactics to restrict the disease's spread must be put in place. Unfortunately, current trachoma management efforts fall short.

In mice that were transcervically infected with *Chlamydia trachomatis*, live bacteria could induce the expression of PD-L1 in uterine epithelial cells and lymph node dendritic cells; but this was not maintained after the primary infection was cleared. The expression of PD-1 in infected mice was not different from the uninfected group until the memory phase, where CD8+ T cells within draining lymph nodes were highly positive for PD-1 [77], in agreement with the findings where *Mtb* antigen-specific memory T cells remained active while expressing PD-1.

The infection with *Chlamydia muridarum* was explored, and by blocking TIM-3 and PD-L1 using mAbs at the beginning of the acute phase, increased macroscopic inflammation in the oviduct was produced. It progressed to hydrosalpinx and oviduct luminal dilatation, and microscopic inflammatory mononuclear infiltrates were higher in the treated group [78]. This finding is consistent with the overall concept of the role of the checkpoint molecules in minimizing the inflammatory damage caused by excessive and prolonged immune responses.

The tryptophan-degrading activity of IDO-1 is increased in neutrophils by *Chlamydia* infection, and the addition of IFN-γ does not affect *C. trachomatis* growth. This situation contrasted with findings in HeLa human cervical epithelial cells, where IFN-γ induced IDO-1 activity correlated with growth inhibition [79]. These findings suggest that the effect of the co-inhibitory molecules in different cell subsets can play different roles; so, the need for research into the signaling pathways in the cells that express these molecules continues to grow.

Conclusions

The need for new therapeutic approaches to treat chronic bacterial infections, in which underlying mechanisms are not fully understood, leads to the idea that modulating the interaction between co-inhibitory molecules can improve T cell response by "releasing the brake" of exhaustion. Nonetheless, existent evidence has demonstrated that the expression of PD-1 or CTLA-4, even during the chronic stage of infection, means that T cells are active, and their function is to prevent an exacerbated inflammatory response and tissue damage while controlling bacterial growth.

There is a need for additional studies to understand the role of known co-inhibitory molecules other than PD-1, PD-L1, and CTLA-4. It is also necessary to systematically investigate the local and systemic expression of these molecules, the signaling pathways related to these co-inhibition molecules and how they are related to co-stimulatory pathways, the result of using mAbs to disrupt their signaling pathways, and the influence of pro-inflammatory and anti-inflammatory cytokines; and these must be further studied and compared to published cancer reports.

This review did not consider the expression of these molecules in cells aside from the immune system; however, they actively participate in influencing the microenvironment by secreting mediators like chemokines and anti-microbial peptides. Moreover, when considering these mechanisms, bacterial virulence factors should be considered as inductors of the expression of co-inhibitory molecules and as an evasion mechanism. Aside from genetic traits in human hosts, this could explain why some infected hosts can live with latent infection while others develop active disease under certain conditions.

This review made it clear that more research needs to be done on the mechanisms underlying the induction and outcome of co-inhibitory molecule expression and the interaction at the signaling pathway level in bacteria-host cell interaction.

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Authors' contributions

MLRC conceptualized and wrote this manuscript. MCSC critically revised this manuscript.

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