

Review

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# The immune regulation of PD-1/PDL-1 axis, a potential biomarker in multiple sclerosis

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## Abstract

Multiple sclerosis is an autoimmune disease characterised by a chronic inflammation within the central nervous system. In the last ten years, studies on multiple sclerosis have been concentrated on the discovery of new biomarkers of disease and potential therapeutic targets. In chronic infection or in cancer, the immune system response is faulty and maintained in a condition defined as T-cell exhaustion induced by expression of co-inhibitory receptors. The PD-1/PDL-1 pathway is demonstrated to be the main one responsible for promoting T-cell exhaustion, and immunotherapies targeting PD-1 or PDL-1 have shown beneficial clinical outcomes in several tumours and chronic diseases. Contrarily, transcriptional T-cell exhaustion signature and high expression of co-inhibitor receptor PD-1 are associated with favourable prognosis in multiple sclerosis and other autoimmune diseases. Several studies have clearly demonstrated PD-1 has a dual role in immune self-tolerance: to constrain autoreactive T cells in anergic condition and to protect the tissue from the damage caused by the activation of endogenous autoreactive T cells. Consequently, immune checkpoint inhibitor therapies that target inhibitory receptors in cancer cause an exacerbation of autoimmune diseases. This review describes the roles of the PD-1/PDL-1 pathway in cancer and autoimmune diseases, especially in multiple sclerosis, and how manipulating PD-1 can be a therapeutic approach in multiple sclerosis.

**Keywords:** T-cell exhaustion, inhibitory checkpoints pathways, PD-1/PDL-1 axis in autoimmune disease, multiple sclerosis, immune checkpoint inhibitor treatments, multiple sclerosis biomarkers



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## T-CELL EXHAUSTION

The word “exhaustion” originates from the Latin “exaurire” and was used for the first time to explain a mechanism for silencing antiviral T-cell response during a Lymphocytic Choriomeningitis Virus infection (LCMV)<sup>[1]</sup>. Antigen-specific CD8 T cells remove viral infection by killing infected cells and the production of antiviral cytokines such as interferon gamma (IFN $\gamma$ ). The damping of immune response in LCMV was associated with two mechanisms silencing the CD8 Cytotoxic T Lymphocytes (CTLs) response: the depletion of nucleoprotein-specific CD8 T cells and the persistence of exhausted glycoprotein-specific CD8 T cells unable to kill virus-infected cells and release antiviral cytokines<sup>[1]</sup>. Further investigations showed that the exhaustion process suppresses the CD8 antiviral activity by the hierarchical loss of T cell function<sup>[2]</sup>. Proliferation, release of IL-2 and cytotoxicity were lost at an early stage of exhaustion, followed by tumor necrosis factor alpha (TNF $\alpha$ ) production and, at the severe late stage, IFN $\gamma$  production. CD4 T helper cells (Th cells) drive the fate of CD8 T-cell responses in chronic viral infections. Mice with transient depletion of CD4 T cells before infection with chronic strains of LCMV develop CD8 T-cell exhaustion and high viral load compared with non-treated mice<sup>[3]</sup>. Th cells are necessary for the generation of stable and functional CD8 memory cells<sup>[4-6]</sup>. During a chronic infection, CD8 T cells develop an exhaustion phenotype that produces a state of immunosuppression in the absence of CD4 T cells<sup>[7]</sup>. The exhaustion process induces low levels of Th cells<sup>[8-10]</sup> and affects CD4 T cell functions with loss of proliferation and IL-2 and TNF $\alpha$  production<sup>[11]</sup>. Moreover, CD8 T cell and B cell response was restored when functional LCMV-specific CD4 T cells were transfected in LCMV chronically infected mice. PD-1 expression increased in LCMV-specific CD4 T cells by two weeks after transfer in chronically infected mice and programmed death 1 (PD-1) blockade improved the CD4 T-cell activity<sup>[12,13]</sup>. In addition, the rescue of CD8 T cell function in terms of proliferation and cytokine release was greater in mice receiving the combination of PD-1 blockade and Th cells compared with the mice receiving either treatment alone<sup>[12]</sup>.

## INHIBITORY CHECKPOINT PATHWAYS

Cytotoxic T Lymphocytes A-4 (CTLA-4), PD-1 and programmed death ligand 1 (PDL-1) are the first inhibitory checkpoint receptors to be discovered and targeted in cancer immunotherapy and chronic viral infection. The amplitude of T-cell response depends on the activation of co-stimulatory (CD28) or inhibitory receptors after the engagement of T-cell receptor (TCR) with the cognate-peptide-major histocompatibility complex. Co-inhibitory receptors show distinct patterns of expression and different mechanisms of action and signalling.

The knockout CTLA-4 mice has shown a lethal hyperactivation phenotype, confirming that CTLA-4 is a vital inhibitor checkpoint of the immune system. After TCR activation, CTLA-4 upregulates in the CD4 T cells and competes with the co-stimulatory receptor CD28 for its ligands CD80 and CD86, for which CTLA-4 has more binding affinity. The link of CTLA-4 to CD80 and CD86 inhibits T-cell activation. Because antigen-presenting cells and dendritic cells express CD80 and CD86, the suppression of anti-tumour immunity by CTLA-4 is thought to occur in the secondary lymphoid organs as well as in the tumour microenvironment.

PD-1 is an inhibitory receptor that belongs to the CD28 family. The receptor has been detected on activated T lymphocytes, B lymphocytes, dendritic cells, macrophages and natural killer cells after a transcriptional activation<sup>[14]</sup>. PDL-1 is the ligand of PD-1, belongs to B7 family and is present on B lymphocytes, antigen-presenting cells (APC) and tissue cells, including several types of cancer. PD-1 engagement activates the inhibitory phosphatase PP2A and SHP-2 by immune receptor tyrosine inhibitory motif and immune receptor tyrosine switch motif, inhibits T-cell activation and increases T-cell migration within tissues.

T-cell exhaustion in cancer and infectious diseases. T-cell exhaustion has been described in animal models of polyomavirus<sup>[15]</sup> and adenovirus<sup>[16]</sup>, as well as in chronic human infections mediated by human

immunodeficiency virus (HIV)<sup>[17]</sup> and hepatitis B and C virus (HBV, HCV)<sup>[18,19]</sup>. The loss of antiviral activity on CD8 T cells is associated with the upregulation of PD-1 in an animal model of LCMV followed by hierarchy suppression of cyto- and cytotoxic function<sup>[20]</sup>. The CD8 cytotoxic function against infected cells and antiviral cytokines production can be restored by blocking the PD-1/PDL-1 pathway, leading to clearance of infection in LCMV. The PD-1/PDL-1 pathway is the principal regulator of T-cell exhaustion in the animal model of LCMV. Studies on PD-1 in human chronic infections such as Human HIV and HCV have shown an increase of PD-1 on virus-specific CD8 T cells. PD-1 increases in HIV-specific CD4 and CD8 T cells and is directly correlated with the viral load and inversely with CD4 T cell counts. Furthermore, PD-1 increases in patients with HIV progression as compared with patients with long-term progression.

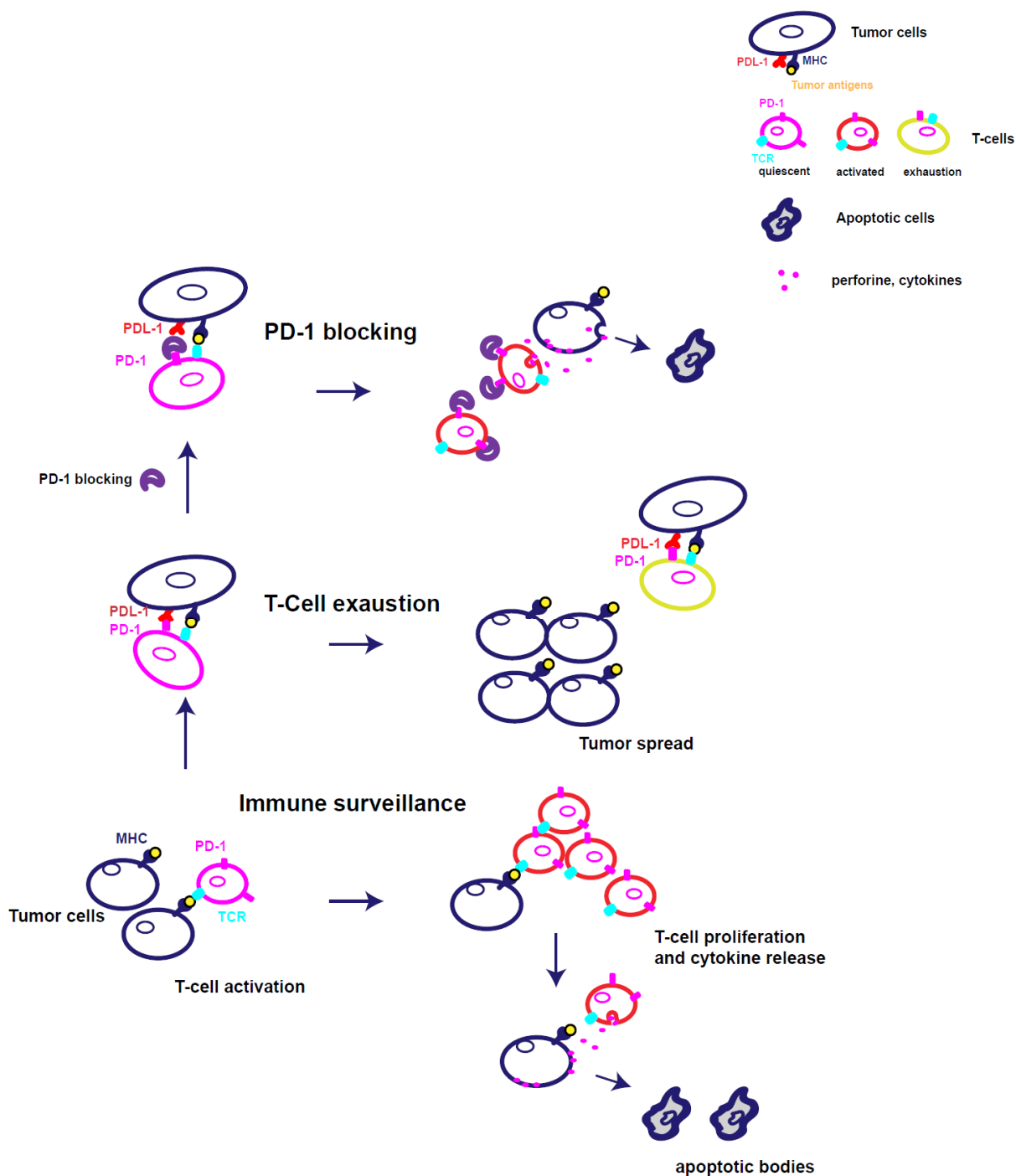
Moreover, T-cell exhaustion suppresses cancer immune-surveillance, leading to tumour spread. Restoring the immune surveillance by blocking PD-1/PDL-1 pathway has been an essential improvement in the cancer treatment. The function of PD-1 and its deregulations are summarised in [Figure 1](#).

Nonetheless, some tumours develop resistance to PD-1 blocking, which is regulated by the tumour microenvironment where infiltrates of regulatory and immune suppressor cells (myeloid suppressor cells, regulatory T cells, immature dendritic cells and immune-suppressive macrophages) reduce the activity of cytotoxic CD8 T cells. Any treatment that induces changes in the levels of hormones and growth factors increases the vulnerability of cancer cells to cytotoxic drugs, which become sensitive to PD-1 treatment. Furthermore, short-term starvation (STS) has been described to reduce levels of insulin-like growth factor 1 (IGF-1) in the lung cancer microenvironment with an increase in the infiltration of immune cells and cytotoxic CD8 T cells<sup>[21]</sup>. The combining of PD-1 blockade treatment with STS boosts the immune system, reducing the tumour size significantly in a mouse model of KRAS-driven lung adenocarcinoma and Lewis lung carcinoma<sup>[21]</sup>. The combination of the two treatments induced in the mice an extended lasting memory response. The immunological study has shown an increase in tumour-infiltrating CD8 and natural killer cells by reducing the proportion of CD4 and B cells. CD8 and CD4 T cells showed a reduction in PD-1 expression. Depletion of CD8 T cells abrogated utterly the effect of the STS and anti-PD-1 treatment, confirming that STS sensitises the lung cancer to CD8 T cells reactivated by PD-1 blocking. The tumour-immune infiltrate treated with anti-PD-1 after STS was analysed with a flow cytometer and presented an increase in the frequency of tumour-specific IFN- $\gamma$ -producing T cells as compared with mice treated with only one agent or vehicle<sup>[21]</sup>.

A selective ablation of PD-1 on myeloid cells or T cells has essentially contributed to understanding the function of PD-1 in the cancer-immunity cycle. Mice with PD-1 ablated only on myeloid cells showed an increase of effector memory T cells and an enhanced response against the tumours. Ablation of PD-1 on myeloid cells changes the tumour microenvironment, skewing the myeloid cell fate toward differentiation of monocytes, macrophages and CD11c<sup>+</sup>MHCII<sup>+</sup> dendritic cells (DC) rather than myeloid suppressor cells and granulocyte/macrophage progenitors. The reduction of myeloid suppressor cells due to PD-1 ablation contributes to restoring the functionality of effector memory T cells and, consequently, an immune response to the tumour<sup>[22]</sup>.

## PD-1/PDL-1 AXIS IN AUTOIMMUNE DISEASE

Autoimmune thyroid diseases (AIDTs) are an organ-specific autoimmune disease that affects 50/100,000 people per year, with a prevalence in females<sup>[23]</sup>. Infiltrating lymphocytes generating follicle structures are described in the thyroid glands in Hashimoto thyroiditis and Grave's disease (GD), the most common AIDTs<sup>[24]</sup>. Interferon signalling and increased expression of PD-1 and M2 macrophages markers were revealed in the transcriptomic analysis of GD glands<sup>[25]</sup>. Thyroid autoimmunity is one of the most common Immune-Related Adverse Events observed after immune checkpoint inhibitors (ICI) treatments in cancer<sup>[26]</sup>.



**Figure 1.** PD-1 inhibitor checkpoint regulates T cell activation during immune surveillance and induces T-cell exhaustion in cancer. (1) PD-1 is expressed on activated T cells and regulates the activity of late differentiate effector cells. The foreign antigens are presented to T cells by the TCR engagement with MHC expressed on dendritic cells, infected cells or tumor cells. Thus, T cells proliferate and differentiate in effector and memory cells. Effector cells kill the foreign antigens express on infected or tumor cells by releasing inflammatory cytokines and cytotoxic granules that induce target-cells to apoptosis. PD-1 is an inhibitory checkpoint able to regulate the T cell activation when the inflammation is resolved. (2) After activation, PD-1 increases on T cells and the link with the ligand PDL-1 reduces the T cell functionality. Thus, T cells are not able to kill the tumor or reduce the viral load and this condition called T-cell exhaustion favours a persistent infection and tumor spread. (3) The blocking of PD-1 with monoclonal antibodies restores T cell function. T cells proliferate and differentiate in effector and memory cells contributing to resolve the infection or tumor. MHC: major histocompatibility complex

The PD-1/PDL-1 axis has been investigated in the peripheral blood and the infiltrating lymphocytes in glands of patients with GD and compared with non-multinodular goitres as non-autoimmune controls and healthy controls (HC)<sup>[27]</sup>. A decrease of naïve as well as an increase of memory and effector subsets of CD4 T cells was observed in GD as compared with the healthy donors (HD)<sup>[27]</sup>. Besides, infiltrating lymphocytes in the gland of GD patients were predominantly effector and memory cells. PD-1 was found higher in GD than HD in CD4 T cells, and it increased in effector memory T cells re-expressing CD45RA (TEMRA), effector and central memory subsets. PD-1 expression increased in infiltrating CD4 and CD8 T cells in infiltrating lymphocytes with predominance on effector and memory subsets. The expression of PDL-1 but not programmed death ligand 2 (PDL-2) was observed in epithelial thyroid follicular cells in the thyroid tissue from GD patients but not in non-multinodular goitres patients<sup>[27]</sup>.

Rheumatoid arthritis (RA) is a chronic progressive inflammatory disorder characterised by damage of articular cartilage and joint destruction<sup>[28-31]</sup>. Environmental, genetic, infectious and hormonal factors can contribute to the pathogenesis of the disease<sup>[32,33]</sup>. The overproduction of TNF $\alpha$  generates inflammation and damages the joints. The interaction of B and T lymphocytes<sup>[34]</sup> with synovial-like fibroblasts and macrophages causes the overproduction of TNF $\alpha$  that induces the production of several inflammatory cytokines, such as interleukin-6 (IL-6)<sup>[35,36]</sup>. Several animal and clinical studies revealed the presence of CD4 T cells in the perivascular cuff and infiltration of CD8 T cells into the tissue. Depletion of T cells or treatment of anti-cytokines that are involved in T-cell activation or promote antigen-presentation reduces inflammation. T helper 17 cells are the primary T cell subsets involved in inflammation and autoimmunity in RA<sup>[37]</sup>. PD-1<sup>-/-</sup> C57BL/6 mice developed arthritis. PD-1 polymorphisms have been reported to be associated with RA<sup>[38,39]</sup>. Expression of PD-1 was detected in synovial T cells and macrophages in patients with RA<sup>[40]</sup>. In the peripheral blood of RA patients, PD-1 was significantly decreased in CD4 T cells ( $P = 0.002$ ) and CD8 T cells ( $P < 0.001$ ) as compared with HC ( $P < 0.05$ )<sup>[41]</sup>. DAS28 score is a measure of disease activity in RA, and PD-1 expression was found inversely correlated with DAS28 scores in RA patients<sup>[41]</sup>. Besides, CRP is an indicator of inflammation and cases with positive CRP detection had a lower proportion of PD-1<sup>+</sup>CD4<sup>+</sup> T cells than those with negative CRP<sup>[42]</sup>.

Systemic Lupus Erythematosus (SLE) is an autoimmune disease generated by the production of antibodies against self-antigens and deposition of immune complexes in different tissues. Inflammation and multisystem disorders characterise the disease<sup>[43]</sup>. The disorder affects mainly women of reproductive age with an incidence of 20-70 cases per 100,000 individuals<sup>[44,45]</sup>. Environmental, genetic and hormonal factors are relevant in the pathogenesis of the disease<sup>[46-48]</sup>. Genetic variations in the immune checkpoint genes such as PD-1, T-cell immunoglobulin domain, mucin domain (TIM) and CTLA-4 increase the susceptibility to develop the autoimmune disease as a consequence of the breakdown of immune tolerance to self-antigens<sup>[49,50]</sup>. Several single-nucleotide polymorphisms (SNPs) have been identified to affect PD-1 function and to contribute to tumours and autoimmune disease<sup>[49,50]</sup>. The frequencies of PD-1 SNPs (PD1.1, PD1.3, PD1.5 and PD1.9) were analysed in SLE patients. The PD1.5 genotype frequency was increased in Iranian, Malaysian and European patients with SLE as compared with healthy donors<sup>[51-53]</sup>. The distribution of PD1.5 C/C, PD1.5 C/T and PD1.5 T/T genotypes versus other genotypes in patients with SLE differed from healthy controls<sup>[53]</sup>. In addition, there were significant differences in the PD1.5 genotypes between patients with renal involvement and neurological involvement and between neurological involvement and HC<sup>[53]</sup>. The allelic analysis revealed that there was a significant association between PD1.5 allele frequency and SLE susceptibility<sup>[53]</sup>.

Type I diabetes (T1D) is caused by autoreactive cells that destroy the insulin-producing beta cells in the pancreatic islet of Langerhans<sup>[54]</sup>. PD-1 and PDL-1 protect from T1D. PD-1 deficiency accelerates the onset and the frequency of T1D in NOD (non-obese diabetic) mice and infiltration of T cells into the islets. PD-1 or PDL-1 but not PDL-2 blockage rapidly induces diabetes in NOD mice with an expansion

of activated glutamic acid decarboxylase (GAD)-reactive cells<sup>[55-59]</sup>. In addition, despite CTLA-4 blockage showing a negative regulation of autoimmune diabetes only in early stages of the life, the PD1-PDL-1 pathway regulated autoreactive T cells throughout the life span of the animal and appeared to be critical for progression of autoimmune diabetes<sup>[59]</sup>. Moreover, polymorphisms that reduce the function of PD-1 are associated with human T1D<sup>[60]</sup>. PD-1 function was also investigated by using a model that mimics the naïve pre-immune repertoire. Fewer islet specific BDC2.5 transgenic naïve CD4 T cells were transferred into prediabetic NOD mice<sup>[61]</sup>. BDC2.5 CD4 T cells accumulated in the pancreas surrounding the islet (peri-insulinitis)<sup>[61]</sup>. When BDC2.5 naïve T cells were preactivated *in vitro* and then transferred into NOD mice, the majority of them accumulated in the pancreas but within the islet (insulinitis), developing severer T1D<sup>[61]</sup>. The majority of BDC2.5 cells differentiate in IFN $\gamma$ -producing cells. Anti-PDL-1 administration caused a conversion from peri-insulinitis to destructive insulinitis<sup>[61]</sup>. PD-1 on BDC2.5 naïve T cells regulate proliferation, C-X-C Motif Chemokine Receptor 3 (CXCR3) expression, infiltration of the pancreas, and release of inflammatory cytokines IFN $\gamma$ , TNF $\alpha$  and IL-2. Moreover, PD-1 but not PDL-1 expressed by BDC2.5 cells is required to suppress proliferation and infiltration of the pancreas<sup>[61]</sup>.

A fusion protein containing a single-chain variable fragment (scFv) of PD-1 antibody (aPD-1), an albumin-binding protein and *Pseudomonas aeruginosa* exotoxin A was used to select and kill PD-1<sup>+</sup> cells<sup>[62]</sup>. The treatment was tried first in animal model of T1D. Depletion of PD-1<sup>+</sup> cells inhibited the development of T1D in NOD mice, reducing the pancreatic infiltration of PD-1<sup>+</sup> cells as compared with the controls. Contrarily, anti-PD-1 was observed to induce a T1D progression in NOD mice, suggesting that PD-1 blocking restores the proliferation and effector function of autoreactive cells. The T1D progression was reduced in mice pre-treated with PD-1 depletion before PD-1 blocking, confirming that PD1 is expressed in autoreactive cells<sup>[62]</sup>.

Multiple Sclerosis (MS) is an autoimmune disease of the central nervous system (CNS). Disease genetic and cellular studies sustain that autoreactive T cells are responsible for CNS damage<sup>[63,64]</sup>. Post-mortem studies showed that T and B cells infiltrate the CNS and, in the long term, develop lymphoid follicles with a functional germinal centre in the meninges and this meningeal inflammation causes white matter demyelination<sup>[65]</sup>. Further investigations demonstrated that inflammatory cytokines and molecules involved in T and B cell development and lymphoid-neogenesis increased in the cerebrospinal fluid (CSF) from post-mortem MS cases with a high level of meningeal inflammation and Gray matter demyelination, as well as in the CSF of patients with MS<sup>[66]</sup> and Gray matter damage at diagnosis<sup>[67]</sup>. Moreover, infiltration of T cells enriched the brain lesions<sup>[68]</sup> and T- and B-depleted therapies reduced activity and progression in MS<sup>[69]</sup>.

IFN $\gamma$  and IL-17-producing CD4 T cells have been defined as the effector populations driving CNS damage. Adoptive transfer of Th1 cells inducing experimental autoimmune encephalomyelitis (EAE) and the cytokine profile of cells isolated from the CNS of mice with acute EAE have shown that Th1 cytokines are released from infiltrating CD4<sup>+</sup> T cells and TNF $\alpha$  is predominantly transcribed by macrophages and microglia<sup>[70-72]</sup>. T-bet is the transcription factor regulating Th1 development and IFN $\gamma$  production, and it is induced by interferon  $\gamma$  transducer and activator of transcription (STAT)-1 signalling pathway during T-cell activation. The role of Th1 in inducing EAE was confirmed in STAT-4 and STAT-6 deficient mice. STAT-4 pathway controls the Th1 differentiation and STAT-4<sup>-/-</sup> mice showed resistance to the development of AEA. Mice deficient in STAT-6, which regulates the differentiation of Th2 cells, develop severer AEA and have more Th1 phenotype<sup>[73]</sup>. The IFN $\gamma$ -producing CD4<sup>+</sup>T cells generate in the cervical lymph nodes and Th1 migration happens 24 h before the onset of neurological signs of EAE<sup>[74]</sup>. Although Th1 cells contribute to EAE, IFN $\gamma$  knockdown mice are predisposed to develop EAE and infiltrates of lymphocytes, macrophages and granulocytes were detected in the CNS<sup>[75-77]</sup>. The results from IFN $\gamma$  knockdown and STAT-1 deficient mice established the contribution of other effector cells to the disease pathogenesis.

Besides, the discovery of IL-23 rather than IL-12 being crucial for EAE development led to evaluating Th17 cells and their transcriptional factor ROR $\gamma$ t in the EAE pathogenesis<sup>[78-80]</sup>. Moreover, Th1, Th17 and Th9 were defined to induce EAE with a different disease phenotype<sup>[79,81]</sup>. In addition to effector cells in EAE<sup>[82]</sup>, the mechanisms of immune regulations, including regulatory B and T cells, and expression of inhibitory receptors were investigated in EAE. CD4 T cells were observed to protect against spontaneous development of CNS autoimmunity in EAE<sup>[83]</sup>, and CD4 regulatory T cells characterised by high expression of CD25 and transcription factor FOXP3 isolated from peripheral blood had a reduced effector suppression function in patients with MS as compared with healthy donors<sup>[84]</sup>. A defect in regulatory B cells was also described to induce EAE and autoimmunity in mice and patients<sup>[85,86]</sup>.

Single-cell transcriptomics of blood and CSF cells isolated from patients with MS and healthy donors revealed that different mechanisms operate in the two compartments<sup>[87]</sup>. Analysis of the data showed that MS affects the cellular composition of the CSF and the transcriptional phenotype of blood cells<sup>[87]</sup>. Blood cells exhibited several transcriptional changes, including induction of activation markers (ICOS), cytokine receptors (IL17RA) and trafficking molecules (PECAM1/CD31 and ITGA5/a5 integrin) in T cells<sup>[87]</sup>.

Contrarily, an enrichment of CD4 T cells with T helper 1 and T follicular helper (Tfh) profiles, regulatory T cells, myeloid lineage cells and late-stage B lineage cells were detected in the CSF<sup>[87]</sup>. Furthermore, Tfh cells expressing PD-1 were observed to correlate with the proportion of plasma cells and showed cytotoxicity and co-inhibitory function. Follicular T helper (Tfh) cells, a subset of T helper cells, are necessary for B cell differentiation and antibody production<sup>[87,88]</sup>. These cells express CXCR5, CD40 ligand and IL-21 as well as high levels of inducible T-cell costimulator (ICOS) and PD-1. They were described to migrate in the germinal centre and to activate B cells. An elevated frequency of circulating Tfh and B cells was identified in MS patients undergoing relapse and Tfh-like cells upregulated during the course of EAE progression<sup>[88]</sup>. In addition, an adoptive cell transfer experiment showed that myelin oligodendrocytes glycoprotein (MOG)-reactive Tfh-like cells induced a worsening of the disease, delaying the remission of EAE *in vivo*<sup>[88]</sup>. Despite the use of PD-1 to identify Tfh cells, the role of PD-1 signalling on Tfh cells is only beginning to be investigated. When PD-1 is engaged by its ligand PDL-1 on follicular B cells, a bystander mechanism is activated and PD-1-expressing Tfh cells are recruited into a special niche inside the GC, even though Tfh PD-1<sup>neg</sup> migrates to the follicle outside of the germinal centre<sup>[89]</sup>. Moreover, both ICOS and PD-1 are requested for maintaining the stringency of affinity-based selection between Tfh cells and antigen-specific cells<sup>[89]</sup>.

The transcriptional signature of CD8 T-cell exhaustion predicted better prognosis in multiple autoimmune diseases<sup>[90]</sup>. Transcriptomes of CD4 and CD8 T cells isolated from a group of patients with active autoimmune diseases were analysed to identify modules of genes with a strong correlation with relapse rate. Modules corresponding to CD4 T-cell co-stimulation were found to correlate with clinical outcomes. In detail, CD4 co-stimulatory receptors, CD2, KAT2B and other surrogate markers were described to increase in MS patients with active autoimmune disease.

The immune regulatory role of PD-1 in MS was suggested by experiments in EAE, a mouse model of MS<sup>[59,91,92]</sup>. Mice in which PD-1 was deleted or the PD-1 pathway was inhibited by blocking the link between PD-1 and its ligand PDL-1 develop a worsening EAE with an increase of infiltrating immune cells, especially CD8 T cells into the CNS<sup>[59,92,93]</sup>. The deterioration of disease in PD-1<sup>-/-</sup> and PDL-1<sup>-/-</sup> mice was related to over production of inflammatory cytokines IFN $\gamma$ , TNF $\alpha$ , IL-6 and IL-17 released by draining lymph node cells during re-stimulation *in vitro* with different concentrations of MOG<sup>[93]</sup>. PDL-1 is rarely expressed in the brains of controls<sup>[94]</sup>. Contrarily, PDL-1 was detected in the majority of lesions expressed from astrocytes and microglia/macrophages with low expression of PD-1 on infiltrating T cells in post-mortem MS brain tissues<sup>[94]</sup>. A recent publication shows that the PDL-1 in dendritic cells improves EAE in

mice. The authors used a hypomethylating agent 5-aza-2'-deoxycytidine, which reduces methylation in a "CPG" island located near the transcription site of *Cd274* gene. The reduced methylation favours the gene transcription and upregulation of PDL-1 in dendritic cells. This effect was observed in DC isolated from EAE mice pre-treated with hypomethylating agent and *in vitro* treatment of bone marrow dendritic cells with the hypomethylating agent. Furthermore, DC isolated from EAE mice pre-treated with 5-aza or bone marrow dendritic cells treated *in vitro* with 5-aza suppressed proliferation and release of inflammatory cytokines such as IL-17 and TNF $\alpha$  when the DC were co-cultured with CD4 T cells isolated from EAE mice. In addition, an inhibition of EAE was observed in mice pre-treated with 5-aza before EAE induction. An increase of PDL-1 and PDL-2 was detected in DC isolated from EAE mice in accordance with the *in vitro* results. Moreover, blocking of PDL-1 but not PDL-2 exacerbated the EAE symptoms, confirming that the link of PDL-1 but not PDL-2 with PD-1 is relevant in the suppression of T cell function by DC<sup>[95]</sup>.

PD-1 depletion was also applied in mice immunised with a peptide of myelin oligodendrocyte glycoprotein as adjuvant to develop EAE<sup>[62]</sup>. After PD-1 depletion, the mice recovered from EAE with a clinical score of one at the end of the experiments compared with the control mice that showed a score four without recovery. Depletion of PD-1 reduced the fractions of PD-1<sup>+</sup>CD4<sup>+</sup> and PD-1<sup>+</sup>CD8<sup>+</sup> T cells but not B cells in the CNS as compared with the controls. Besides, PD-1 depletion did not alter the ability of the treated mice to mount an immune response. The baseline number of PD-1<sup>+</sup> cells in the blood and peripheral lymphoid organs was low, confirming that PD-1 is expressed on autoreactive cells infiltrating target organs<sup>[62]</sup>.

In view of publications giving PD-1 a crucial role in protection against autoimmunity in human and animal models, Jang *et al.*<sup>[96]</sup> investigated how PD-1 controls the activation and accumulation of autoreactive T cells, by constraining them in anergic state. PD-1 is one of the checkpoint inhibitors investigated in self-tolerance and discussed previously in CD8 T cells. Self-reactive cells were deleted during thymic development<sup>[97]</sup> and, of those that survived thymic deletion, only 10%-25% preferentially differentiated into immune suppressive regulatory T cells (Tregs)<sup>[98,99]</sup>. Jiang *et al.*<sup>[96]</sup> demonstrated that PD-1 is required in culling endogenous peripheral high-affinity autoreactive CD4 T cells and protect against autoimmunity<sup>[96]</sup>. The tracking of endogenous autoreactive CD4 T cells showed that more than 90% of autoreactive CD4 T cells remained FOXP3- effectors and were not regulatory T cell precursors, despite the high TCR affinity<sup>[96]</sup>. Instead, self-reactive CD4 T cells acquired cell-intrinsic tolerance through the expression of the immune checkpoint molecule PD-1<sup>[96]</sup>. Monitoring the progeny of individual autoreactive CD4 T cell clones showed that the clones with the greatest expansion burst size and highest TCR affinity expressed high levels of PD-1 and the affinity for the self-antigen induces the expression of PD-1<sup>[96]</sup> and the absence of PD-1 converts this signal when priming with consequent cell activation. A similar mechanism was described to induce the peripheral CD8 T tolerance *in vivo*. The peripheral CD8 T tolerance is induced by resting dendritic cells and depends on activation of PD-1 and CTLA-4 pathways<sup>[100]</sup>.

Several studies have analysed the gene expression and protein levels of PD-1 and PDL-1 in MS, focusing on delineating any correlation with disease susceptibility or risk of progression in MS. *PD-1* gene polymorphism has been investigated in MS, and the PD 1.3 SNP has been reported to correlate with progression of the disease, demonstrating that human polymorphisms that reduce PD-1 activity increase the risk of disease. Furthermore, a significant reduction in PD-1 expression was observed in patients with mutation as compared with donors with wild-type phenotype. Furthermore, patients bearing the mutant allele showed a lower suppression of IFN $\gamma$ -producing CD4 T cells after aCD3-PD-1-microbead stimulation compared with healthy donors<sup>[101]</sup>. In addition, PD-1 and PDL-1 expression in peripheral blood mononuclear cells reduced in a cohort of patients with MS as compared with healthy donors<sup>[102]</sup>. The association of three PD-1 SNPs, namely PD-1.3, PD-1.5 and PD-1.9, with MS and disease outcome were investigated in a cohort of 203 patients with a diagnosis of relapsing-remitting and secondary-progressive MS showing any association with MS risk<sup>[103]</sup>. The expression of inhibitory receptor genes,

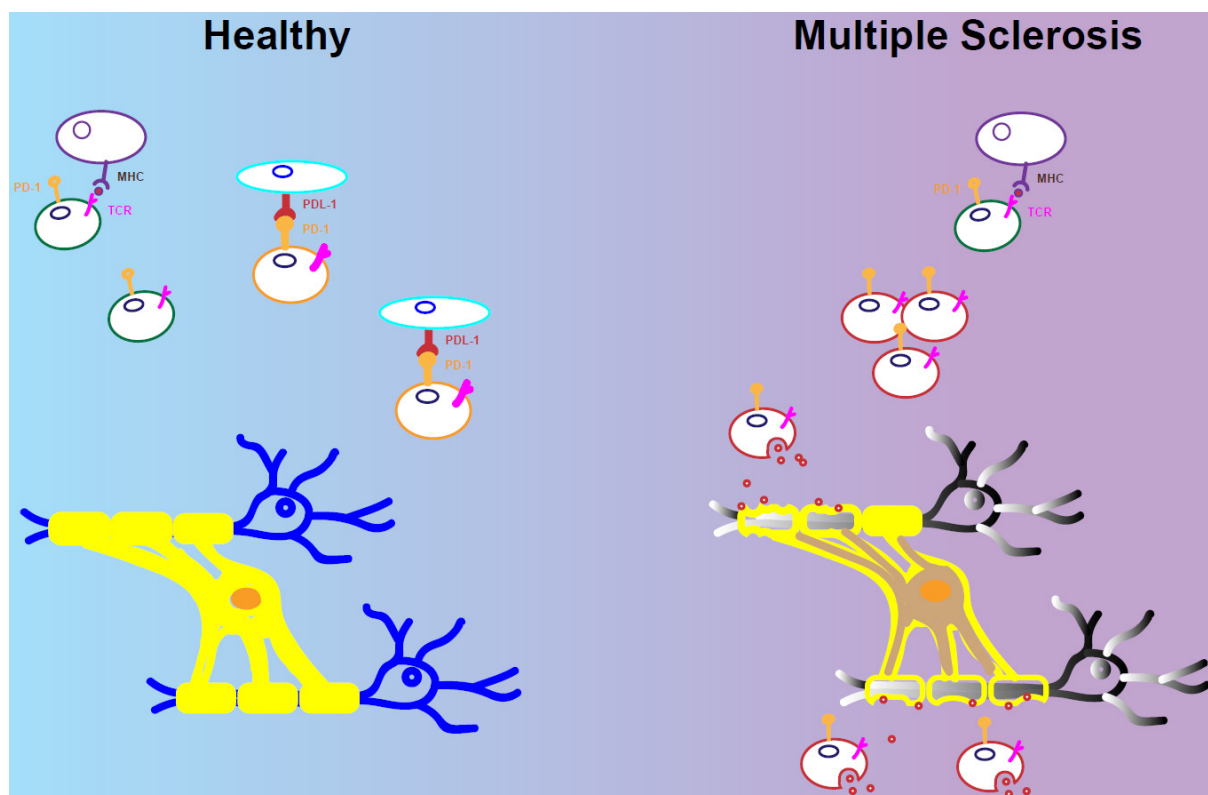


including *CTL-4*, *PD-1* and *TIM-3*, decreased in patients with MS as compared with healthy controls<sup>[104]</sup>. *PD-1* is usually the most downregulated gene among the investigated inhibitors<sup>[104]</sup>. *PD-1* was analysed on cytotoxic CD8<sup>+</sup>CD57<sup>+</sup>T cells in the peripheral blood of patients with relapsing–remitting MS and in T cells infiltrating the brain tissue in post-mortem MS cases. *PD-1* increased in CD8<sup>+</sup>CD57<sup>+</sup>T cells in patients with stable disease and decreased in active-relapsing MS compared with healthy donors<sup>[105]</sup>. *PD-1* was also found to increase in CD4 and CD8 T cells in MS patients early after autologous hematopoietic stem cell transplant<sup>[106]</sup>. A study of long-term immune reconstitution in MS patients after autologous hematopoietic stem cell transplant demonstrated that an early expansion of CD8<sup>+</sup>PD-1<sup>+</sup>T cells and CD19<sup>+</sup>PD-1<sup>+</sup>B cells is associated with favourable neurological outcomes<sup>[106]</sup>. *PDL-1* was also investigated in post-mortem MS brain tissue. In MS lesions, glial cells with elevated *PDL-1* and *PD-1* expression were found absent in many infiltrating CD8 T cells<sup>[94]</sup>. Moreover, *PDL-2* but not *PDL-1* is expressed in human brain endothelial cells under basal culture conditions whilst both are upregulated under inflammatory condition<sup>[107]</sup>. *PDL-1* or *PDL-2* blockade lessens CD8 and CD4 T cell transmigration and CD8 T cells response<sup>[94]</sup>. Furthermore, *PDL-1* is undetectable in the brain endothelium in normal tissues and MS lesions, even though *PDL-2* is detectable in all blood vessels in normal brain tissue and in 50% of MS lesions<sup>[94]</sup>.

## MULTIPLE SCLEROSIS DIAGNOSIS AFTER CANCER IMMUNOTHERAPY

ICI treatments are immunotherapies engaged in restoring the immune response to tumour or viral infection by blocking the inhibitory pathways mediated by *CTLA-4* and *PD-1*. ICI treatments have induced neurological immune-related adverse events. Patients with an MS history developed relapses after ICI treatment for melanoma<sup>[108,109]</sup> and a biopsy of the lesions revealed acute/inflammatory demyelination without any evidence of tumour cells<sup>[109]</sup>. A comparative functional profiling of myelin-reactive T cells of patients after ICI- treatment and 14 age/sex-matched patients with MS and healthy controls was performed. Myelin-reactive T cells isolated from ipilimumab-treated patients and MS patients showed a similar autoimmune response to myelin antigen but distinct from healthy controls. That confirmed that ICI treatment causes a reactivation of self-antigen cells in MS patients. The lack of outcomes to the ICI treatment in tumour is associated with an effect on the neurological condition. A case reported maintaining stable MS during ipilimumab treatment for melanoma that did not respond to the therapy, and the patient died from metastatic melanoma<sup>[110]</sup>.

Furthermore, a 29-year-old man with metastatic melanoma underwent two cycles of ipilimumab before developing MS. The TCR repertoires of tumour-infiltrating T cells isolated from the primary melanoma and those of T cells isolated in two CSF samples, five and thirteen months after the second course of ipilimumab therapy, were analysed and compared. Distinct clonotypes of CD4 and CD8 T cells in the melanoma and the CSF were identified, demonstrating that the protective antitumor response and the anti-CNS response target different antigens<sup>[111]</sup>. Outcomes of MS relapse after ICI treatment were reported in a meta-analysis study including the published literature, the analysis of food and the drug administration adverse event reporting system database and a detailed case<sup>[112]</sup>. Fourteen cases were identified with MS, of which eight had a reported history of MS. All patients presented rapid disease progression, and two of them died from severe MS after ICI treatment<sup>[112]</sup>. The median age of MS diagnosis was 52.5 years, and ICI treatment was used as immunotherapy in several types of cancer: melanoma, non-small cell lung carcinoma, pleural mesothelioma, renal cell carcinoma and colorectal cancer<sup>[112]</sup>. ICI treatments such as nivolumab, ipilimumab, pembrolizumab and atezolizumab have caused MS relapse. In addition, Isitan and Wesley<sup>[113]</sup> described the case of a 49-year-old woman with a history of relapsing–remitting MS reported to develop a severe progressive MS after atezolizumab (monoclonal antibody targeting *PD-1*) therapy for metastatic colonic adenocarcinoma. The woman died after her first dose of atezolizumab. Although ICI therapy has given beneficial outcomes in cancer and infectious diseases, this treatment has shown neurological side effects in patients with MS history, inducing a rapid worsening of neurological conditions. The examined cases showed a worsening of the conditions associated with the activation of T



**Figure 2.** Immune checkpoints inhibitor treatment induces reactivation of multiple Sclerosis. PD-1 has been described on autoreactive T cells and TFH cells in multiple sclerosis. The link of PD-1 with the ligand PDL-1 controls T cell activation maintaining tolerance (Healthy). The blocking of PD-1 breaks the tolerance by reactivation of antigen-specific cells and causing damage of the target-cells with persistent inflammation in the CNS (Multiple Sclerosis). Contrary, depletion of PD-1<sup>+</sup> T cells eliminates activated antigen-specific cells reducing the T cell infiltrates and inflammation in the target-organ (PD-1 blocking). TFH: T Follicular helper cells

cells recognising self-antigen in the CNS. The relevance of PD-1 blocking and depletion of PD-1<sup>+</sup> cells in the pathogenesis of multiple sclerosis is shown in [Figure 2](#).

## CONCLUSION

PD-1 is an immune checkpoint inhibitor demonstrated to reduce the immune system response in cancer and chronic infection. Recent investigations have highlighted the dual role of PD-1 in immune tolerance, and the loss of PD-1 causes autoimmune diseases. Depletion of PD-1<sup>+</sup> T cells has given beneficial effect in autoimmune disease, slowing down the inflammation and disease progression. A decrease of PD-1 is predisposed to autoimmunity, as described in experiments of PD-1 blocking or knockout in mice. PD-1 could be a target of immunotherapies in MS, although further investigations are required to define the role of PD-1 in MS. The majority of information has been derived from animal models and sporadic studies in humans. To this purpose, expression and levels of PD-1 and PDL-1 in the peripheral blood, CSF and post-mortem MS brain tissues and the correlation of their levels with risk of MS disease and inflammation could make relevant contributions for considering PD-1 a target in MS immunotherapies.

## DECLARATIONS

### Authors' contributions

The author contributed solely to the article.

### Availability of data and materials

Not applicable.

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### Conflicts of interest

The author declared that there are no conflicts of interest.

### Ethical approval and consent to participate

Not applicable.

### Consent for publication

Not applicable.

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