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REVIEW

Emerging Role of PD-1 in the Central Nervous System and Brain Diseases

Junli Zhao[1] · Alexus Roberts[1,2] · Zilong Wang[1] · Justin Savage[3] · Ru-Rong Ji[1,3,4]

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Abstract Programmed cell death protein 1 (PD-1) is an immune checkpoint modulator and a major target of immunotherapy as anti-PD-1 monoclonal antibodies have demonstrated remarkable efficacy in cancer treatment. Accumulating evidence suggests an important role of PD-1 in the central nervous system (CNS). PD-1 has been implicated in CNS disorders such as brain tumors, Alzheimer's disease, ischemic stroke, spinal cord injury, multiple sclerosis, cognitive function, and pain. PD-1 signaling suppresses the CNS immune response *via* resident microglia and infiltrating peripheral immune cells. Notably, PD-1 is also widely expressed in neurons and suppresses neuronal activity *via* downstream Src homology 2 domain-containing protein tyrosine phosphatase 1 and modulation of ion channel function. An improved understanding of PD-1 signaling in the cross-talk between glial cells, neurons, and peripheral immune cells in the CNS will shed light on immunomodulation, neuromodulation, and novel strategies for treating brain diseases.

✉ Junli Zhao
junli.zhao@duke.edu

✉ Ru-Rong Ji
ru-rong.ji@duke.edu

[1] Department of Anesthesiology, Duke University Medical Center, Durham 27710, USA

[2] Department of Biology, Duke University Medical Center, Durham 27710, USA

[3] Department of Neurobiology, Duke University Medical Center, Durham 27710, USA

[4] Department of Cell Biology, Duke University Medical Center, Durham 27710, USA

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Introduction

Programmed cell death protein 1 (PD-1, also known as PDCD1 and CD279) is a cell surface receptor which contains 288 amino-acids and is widely expressed in immune cells (T cells, B cells, natural killer cells, dendritic cells, and macrophages) and other cell types (microglia and neurons) (Table 1). In 1992, PD-1 was initially found by the Honjo group at Kyoto University during screening for genes involved in apoptosis [1]. Over the ensuing decades, it has become clear that PD-1 is a negative regulator of immune responses [2, 3] (Fig. 1). PD-1 binds two ligands, PD-L1 (B7-H1) and PD-L2 (B7-DC) [4–7]. PD-L1 is expressed on a variety of hematopoietic and non-hematopoietic cells [8–11]. PD-L2 is mainly restricted to antigen presenting cells (APCs) [8, 12]. Binding to either of these ligands, PD-1 signaling regulates the immune response by down-regulating the immune system and promotes self-tolerance by suppressing T cell inflammatory activity. This inhibitory signaling through the PD-1 pathway is an important mechanism underlying many physiological and pathological conditions. Physiologically, the PD-1 signaling pathway regulates T cell activation, T cell tolerance, and immune hemostasis [13]. Perturbation of the PD-1 pathway can profoundly impact host physiology [14, 15]. Pathologically, PD-1 and its ligands are strongly expressed during many chronic diseases, especially in cancer [16, 17]. In 2002, Minato *et al.* found that *Pdcd1* gene deletion inhibited tumor growth in mice. In 2010, the first clinical trial of anti-PD-1 antibody (BMS-936558/ONO-4538) was launched in Japan for cancer

Table 1 Expression of PD-1 in various tissues and cells

Tissue or cell with PD-1 expression	Level of expression	Function of expression	References (methods)
B-cells	Low expression in peripheral blood under normal conditions	Inhibits B-cell activation, proliferation, and differentiation	[100] (FC, IF), [101], [102] (FC, RT-PCR)
Dendritic cells	Low expression under normal conditions	Restricts T-cell activation and lowers innate immunity	[103] (FC), [104] (FC, RT-PCR)
DRG sensory neurons	Expressed in DRG sensory neurons as well as in axons	Interacts with PD-L1 to modulate pain and lower sensitivity	[19] (IF, ISH, WB), [95, 105] (ISH, IF, PLA, co-IP)
Hippocampal neurons	Expressed in hippocampal CA1 and CA3 neurons, low expression in DG neurons	Regulates neuronal excitability, synaptic transmission, plasticity, and memory	[92] (IF, ISH)
Macrophages	Low expression under normal conditions	Functions as a control mechanism for systemic immune responses through redirection or delays	[31] (FC, IF), [106] (FC, RT-PCR), [107] (FC)
Microglia	Low expression under normal conditions	Regulates the inflammatory reaction after injury or infection	[23] (RT-PCR, IF)
NK cells	Low expression under normal conditions	Prevents NK cell activation and cytotoxicity in specific situations	[108] (FC), [109] (FC, IHC)
Retinal ganglion cells	Expressed in almost all adult retinal ganglion cells	Promotes apoptosis, which is necessary for proper maturation	[110] (IF, IHC, WB, RT-PCR), [111] (IF, WB, RT-PCR)
Spinal cord	Expressed in spinal neurons, primary afferent terminals, and microglia	Regulates pain, opioid analgesia and tolerance, GABAergic neurotransmission	[95] (IF), [24, 95] (ISH)
T-cells	Highly expressed in activated T-cells	Functions as an immune checkpoint receptor	[13, 27, 112] (FC), [113] (IF, FC), [114] (FC, PCR)

Abbreviations FC, flow cytometry; IHC, immunohistochemical analysis; IF, immunofluorescence; ISH, *in situ* hybridization; PCR, polymerase chain reaction; RT-PCR, reverse-transcription-polymerase chain reaction; WB, Western blotting; PLA, proximity ligation assay; co-IP, co-immunoprecipitation; DRG, dorsal root ganglion; NK cells, natural killer cells.

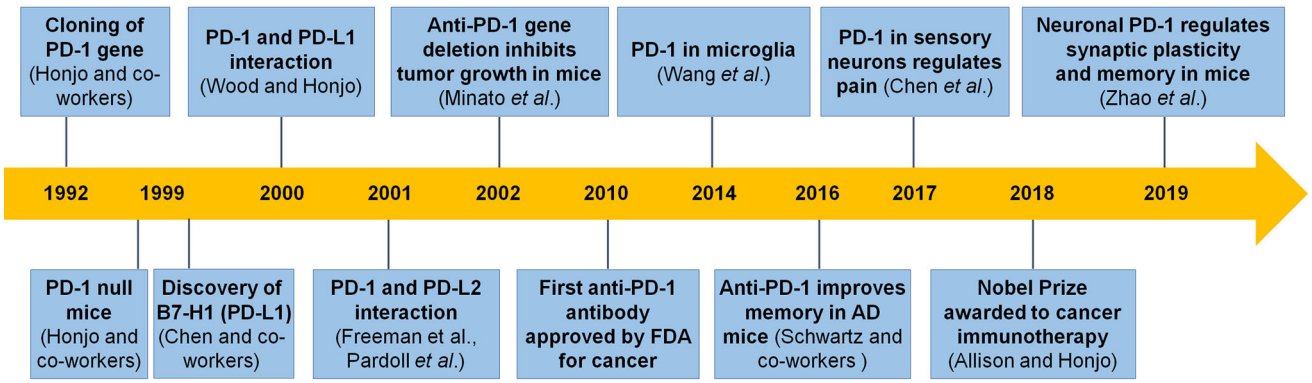

Fig. 1 Timeline for major events leading to the development of PD-1 functions and PD-1-based immunotherapy.

treatment (Fig. 1). Since 2014, several anti-PD-1 monoclonal antibodies such as Nivolumab (Opdivo), Pembrolizumab (Keytruda), and Cemiplimab-rwlc (Libtayo) have been approved by the FDA. Given the success of the emerging immunotherapy with anti-PD-1 and anti-CTLA4 (cytotoxic T-lymphocyte-associated protein 4) monoclonal antibodies in cancer treatment, the 2018 Nobel Prize in Physiology or Medicine was awarded to James P. Allison and Tasuku Honjo for their discovery of cancer therapy by inhibition of negative immune regulation (Fig. 1).

In addition to the prominent role of PD-1 in the immune system, accumulating evidence also suggests an activating

role of PD-1 signaling in both the central nervous system (CNS) and the peripheral nervous system (PNS) (Figs. 2–5). PD-1 reduces neuroinflammatory responses and may also regulate neuronal activity in several CNS diseases, such as brain tumors, Alzheimer's disease, stroke, chronic pain, multiple sclerosis, and cognitive deficits [18, 19]. The mechanisms underlying the actions of PD-1 in these disease conditions are multifaceted. First, the recent progress in demonstrating peripheral immune cell recruitment to the CNS under pathological conditions challenges the historical view of CNS immune privilege. Functional lymphatic vessels in the meninges have recently been discovered that provided a direct drainage pathway for PD-1^+ immune cells from the cervical lymph nodes into the brain [20, 21]. Thus, PD-1^+ immune cells such as T cells may play a role in the CNS similar to that in the peripheral immune system. Second, PD-1 is expressed by macrophages as well as microglia in the spinal cord and brain [22, 23]. Under CNS disease conditions such as brain trauma and spinal cord injury, brain resident PD-1^+ microglia are activated in the spinal cord and brain and PD-1^+ macrophages are also recruited to the CNS, where these microglia and macrophages undergo substantial phenotypic changes to regulate neuroinflammation and disease progression [23]. Finally, accumulating evidence demonstrates that PD-1 is also expressed by CNS neurons and that PD-1 signaling in neurons regulates neuronal excitability, synaptic transmission, and plasticity *via* PD-1/

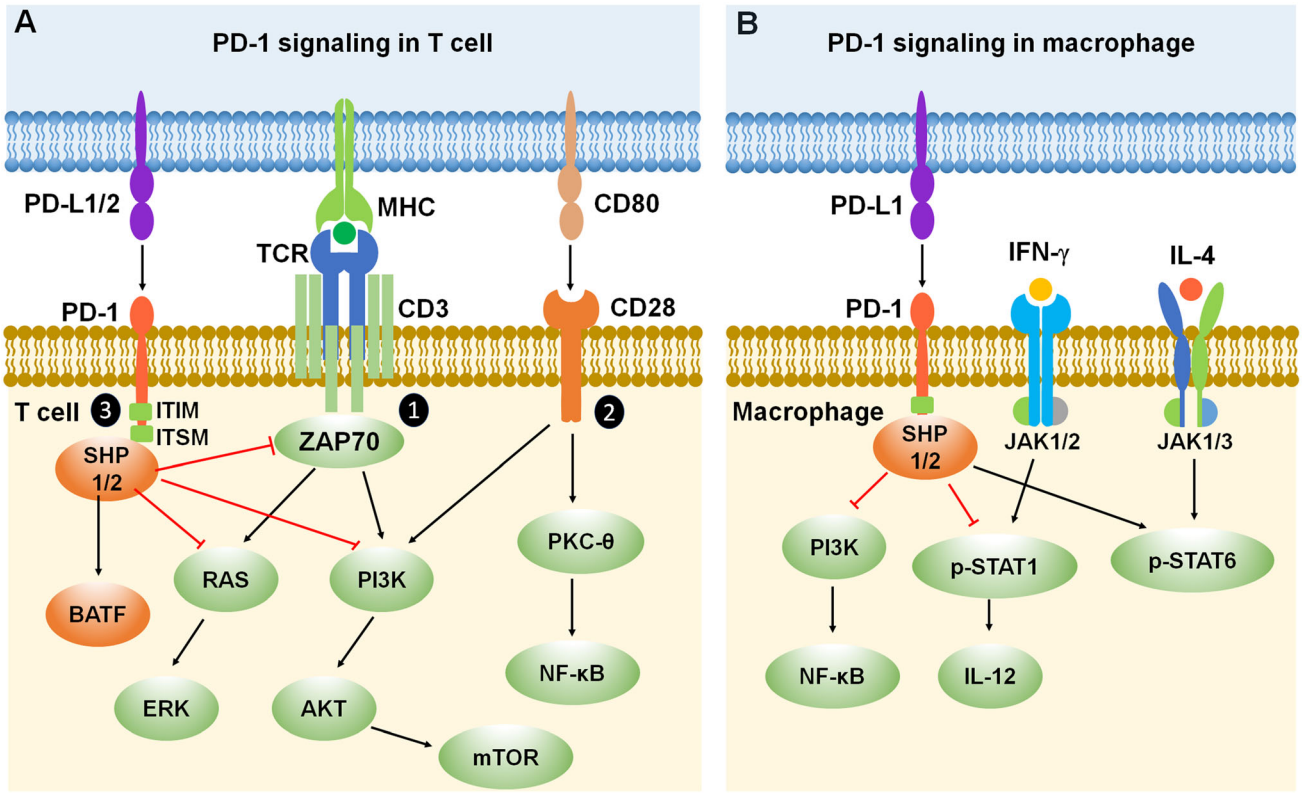

Fig. 2 PD-1 signaling in T cells and macrophages. **A** Mechanisms of PD-1 signaling in T cells. PD-1 inhibits T cell function by recruiting phosphatases SHP-1/SHP-2 to the ITIM/ITSM domain in the PD-1 tail and increasing the expression of transcription factor BATF. In addition, PD-1 inhibitory signaling antagonizes positive T cell signaling events triggered by (1) TCR interacting with MHC and (2) CD28 interacting with CD80. (3) PD-1 signaling inhibits ZAP70 and the RAS-ERK and PI3K-AKT-mTOR signaling pathways. **B** Mechanisms of PD-1 signaling in macrophages. PD-1 inhibits macrophage function by recruiting phosphatases SHP-1/SHP-2 to the ITIM/ITSM domain in the PD-1 tail, leading to inhibition of the PI3K-NF-κB signaling pathway. Moreover, PD-1 signaling suppresses IFN-γ-activated M1 macrophage polarization by reducing the phosphorylation of STAT1 and the secretion of IL-12, while promoting IL-4-activated M2 macrophage polarization by increasing STAT6 phosphorylation. Red lines ending in a bar represent inhibitory signaling, and black arrows indicate positive signaling. Abbreviations: PD-1, programmed cell death protein 1; PD-L1/2, PD-1 ligands, PD-L1 (B7-H1) and PD-L2 (B7-DC); ITIM, immunoreceptor tyrosine-based inhibitory motif; ITSM, immunoreceptor tyrosine-based switch motif; SHP, Src homology 2 domain-containing protein tyrosine phosphatase; BATF, basic leucine zipper ATF-like transcription factor; MHC, major histocompatibility complex; TCR, T cell receptor; CD, cluster of differentiation; ZAP70, zeta-chain-associated protein kinase 70; RAS, a small GTPase encoding *RAS* (retrovirus-associated DNA sequences); ERK, extracellular signal-regulated kinase; PI3K, type I phosphatidylinositol 3-kinase; AKT, serine/threonine-specific protein kinase; mTOR, mammalian target of rapamycin; PKC-θ, protein kinase C theta; NF-κB, nuclear factor kappa B; p-STAT1/6, phosphorylated signal transducer and activator of transcription 1/6; IL, interleukin; IFN-γ, interferon gamma; JAK, Janus kinase.

SHP-1 signaling and downstream modulation of ion channels [19, 24].

The role of the PD-1 pathway in the immune system has been elegantly discussed in a number of reviews [25, 26]. In this review, we focus on the diverse roles of PD-1 signaling in the context of the CNS, including in physiological cognitive function as well as pathological conditions such as brain tumors, Alzheimer's disease, stroke, spinal cord injury, multiple sclerosis, and pain (Table 2). We further discuss how this knowledge can be applied to understanding how the PD-1 pathway can modulate the treatment of CNS diseases. And finally, we consider the challenges and opportunities for utilizing the PD-1 signaling pathway for immunotherapies and neurotherapies in CNS disease conditions.

Immune Modulation of CNS Disorders by PD-1

PD-1 Signaling in Immune T Cells, Macrophages, and Microglia

PD-1 is widely expressed by immune cells and its signaling pathway is best characterized in activated T cells [27]. Activated T cells receive three signals from APCs during cytokine production, proliferation, differentiation, apoptosis, and survival (Fig. 2A). Signal one consists of TCR-CD3 (T cell receptor and cluster of differentiation 3) and its co-receptor (CD4 or CD8) binding to the major histocompatibility complex (MHC), and subsequently activating the co-receptor associated lymphocyte-specific protein tyrosine kinase (LCK). LCK phosphorylates the intracellular portions of the CD3 complex and creates a docking site for zeta-chain-associated protein kinase 70 (ZAP70), which is expressed near the surface membrane of T cells and plays a crucial role in T-cell signaling. ZAP70 starts multiple signaling events through activation of the RAS-ERK (extracellular signal-regulated kinase) and PI3K-AKT (type I phosphatidylinositol 3-kinase to serine/threonine-specific protein kinase) pathways. Signal two is composed of CD80-CD28 interactions between APCs and T cells. LCK phosphorylates the CD28 intracellular domain, providing a docking site for the PI3K complex. PI3K then generates phosphatidylinositol-(3,4,5)-trisphosphate, activating downstream kinases including AKT, which enhances proliferation and survival through the mammalian/mechanistic target of rapamycin (mTOR) pathway. CD28 activation further activates protein kinase C theta (PKC-θ) and subsequent activation of the nuclear factor kappa B (NF-κB) pathway. Signal three is a result of PD-1 signaling which serves as an antagonist of the two activated pathways noted above. PD-1 has two tyrosine-

Table 2 Brain diseases and other conditions influenced by PD-1 signaling

Disease or condition	Resources	Role of PD-1	References
Alzheimer's disease	Human patients	Decreased PD-1 expression decreases amyloid plaques in some studies but not others	[64, 65, 67, 115]
	Mice		
Glioblastoma	Human patients	Tumor cells interact with PD-1 through the PD-l/PD-L1 axis to increase PD-1 expression, which then decreases T-cell production	[116–119]
	Mice		
Melanoma	Human patients	Metastatic tumor cells interact with PD-1 through the PD-l/PD-L1 axis to increase PD-1 expression, which then decreases T-cell production	[120–123]
	Mice		
Multiple sclerosis	Human patients	Increased PD-1 expression correlated with disease remission due to the PD-1/PD-L1 pathway limiting the immune response	[84, 85, 124, 125]
	Mice		
Memory	Mice	PD-1 deficiency or blockade in brain improves learning and memory	[92]
Pain	Human patients	The PD-1/PD-L1 axis has an analgesic effect by suppressing peripheral neuronal excitability and spinal synaptic transmission, thereby reducing pain	[19, 95, 98, 126–128]
	Mice		
Spinal cord injury	Mice	PD-1 is highly expressed after spinal injury to restrict the inflammatory response	[23, 81]
Stroke	Human patients	Increased PD-1 expression linked to reduced post-stroke inflammation, but the PD-1 ligands PD-L1 and PD-L2 play distinct roles in stroke	[22, 71–74]
	Mice		

based signaling motifs in its cytoplasmic domain: an immunoreceptor tyrosine-based inhibitory motif (ITIM) and an immunoreceptor tyrosine-based switch motif (ITSM), both of which are essential for PD-1 function. When engaged with PD-L1, PD-1 counteracts TCR-CD3 signal transduction and terminates ZAP70 and PI3K phosphorylation by recruiting SHP-1 or/and SHP-2 phosphatases to its tyrosine phosphorylated ITIM and ITSM motifs, affecting downstream signaling pathways including those involving PI3K-AKT and RAS-ERK [28]. In addition, PD-1 inhibits T cell functions by increasing the expression of transcription factors such as basic leucine zipper ATF-like transcription factor (BATF), which further counters effector transcriptional programs [29]. The functional outcome of these effects is decreased T cell activation, proliferation, survival, and cytokine production as well as altered metabolism.

Subsequent studies have shown that, in addition to T cells, PD-1 is also expressed by macrophages and microglia (Figs. 2B and 3A), especially under pathological conditions [30, 31]. Macrophages and microglia have different phenotypes such as M1 and M2, which show nearly opposite functionality in the immune system and CNS. M1 macrophages and microglia are highly pro-inflammatory and effective killer cells. M2 macrophages and microglia, on the other hand, are induced by a variety of stimuli, including interleukin-4 (IL-4) [32]. In addition to M1 and M2 phenotypes, macrophages and microglia must have additional phenotypes for maintaining homeostasis and promoting resolution [33–35]. PD-1$^+$ macrophages play the main role in the peripheral immune system, and brain-resident PD-1$^+$ microglia may serve an analogous function in the CNS. In cancer, there is a higher proportion of M2 macrophages among PD-1$^+$ tumor-associated macrophages *versus* M1 macrophages [31]. Anti-PD-1 therapy stimulates macrophage infiltration into tumors and increases the proportion of M1 over M2 macrophages in tumors. Thus, PD-1 signaling alters the function of macrophages and microglia by affecting the M1/M2 phenotypes during pathological conditions.

The canonical interferon (IFN) regulatory factor/STAT (signal transducer and activator of transcription) signaling pathways activated by IFN-γ promote the formation of M1 macrophages *via* STAT1 activation. In contrast, IL-4 promotes the M2 phenotype *via* STAT6 activation [36]. PD-1 activation induces M2 polarization of macrophages and microglia through decreased STAT1 phosphorylation and increased STAT6 phosphorylation, as well as the down-regulation of crucial downstream NF-κB signaling, which otherwise could be activated by PI3K (Figs. 2B and 3A). Activation of PD-1 also reduces the production of cytokine IL-12 by macrophages and microglia, which further regulates the function of immune cells and affects pathological conditions [37] (Figs. 2B and 3A). In addition, microglial ERK activation is a critical regulator of pro-inflammatory immune responses in many pathological conditions including neuropathic pain, and PD-1 signaling may regulate the microglial ERK signaling pathway in these conditions [38–41] (Fig. 3A). Finally, PD-1 signaling in neurons shares similarities with that in immune cells and glial cells but also shows clear differences by functional interactions with ion channels (Fig. 3B).

Anti-PD-1 Immunotherapy for Brain Tumors and Brain Metastases

Anti-PD-1 immunotherapies have been used as clinical treatments for brain tumors [42–44]. Anti-PD-1 blocking antibodies have been shown by various studies to cause an increase of T cells in the brain and anti-tumor immunity by mobilizing the immune system (Fig. 4A). Although PD-1 blockade has shown positive effects for treating brain tumors and metastases in cancer patients, the clinical efficacy of PD-1 blockades has shown certain limitations and unpredictability. The characteristics of certain brain tumors can make effective treatment difficult. Glioblastoma (GBM) is the most common and aggressive brain tumor diagnosed in adults but <10% of GBM patients show a long-term response to anti-PD-1 treatment [45]. One of the limiting factors is the blood-brain barrier (BBB), which makes it difficult for anti-PD1 antibodies such as nivolumab to access the tumor. Combining anti-PD-1 antibodies with BBB peptide shuttles enhances delivery of the drug to the brain and efficiently eliminates brain tumor cells [46]. Another limitation of PD-1 blockade to treat brain tumors and metastases is the low immunogenic response and immunosuppressive microenvironment in brain tumors [47]. Immunosuppression mediated by the CNS-native myeloid cells in the tumor microenvironment has been linked to poor outcomes in cancer and a reduced response to immunotherapies. A recent study showed that loss of Cx3cr1 (C-X3-C motif chemokine receptor 1) in CNS-myeloid triggers a Cxcl10 (C-X3-C motif chemokine receptor 10)-mediated vicious cycle, promoting brain metastases and immunosuppression [48]. TREM2 (triggering receptor expressed on myeloid cells 2)-positive myeloid cells have also been shown to mediate immunosuppression in the tumor microenvironment, and TREM2 deficiency or administration of a TREM2 antibody increases the efficacy of anti-PD-1 immunotherapy [49, 50]. Thus, it will be necessary to identify potential biomarkers in patients who could obtain the greatest benefit from anti-PD-1 treatment. A recent study in GBM patients treated with anti-PD-1 immunotherapy showed a significant enrichment of PTEN (phosphatase and tensin homolog deleted on chromosome ten) mutations associated with

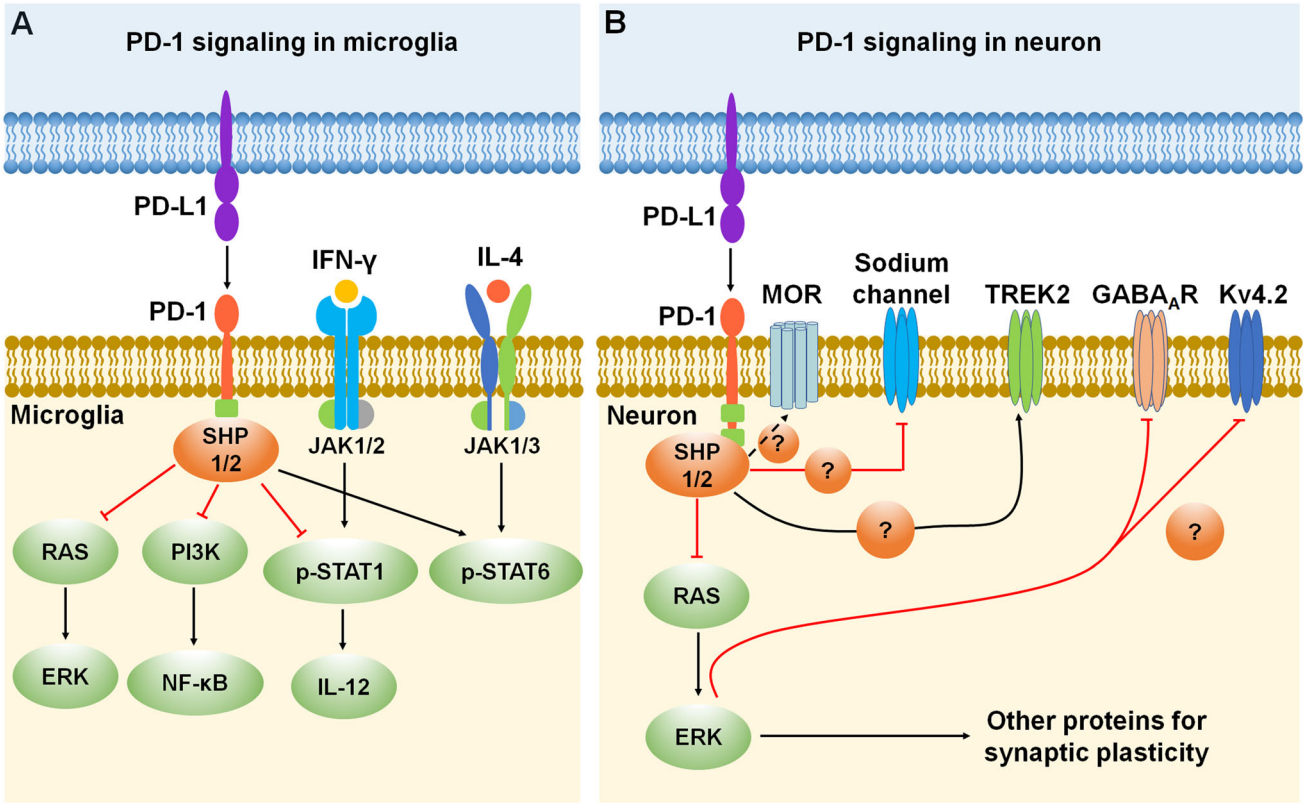

Fig. 3 PD-1 signaling and expression in microglia and neurons. **A** Mechanisms of PD-1 signaling in microglia. PD-1 inhibits microglial function by recruiting phosphatases SHP-1/SHP-2 to the ITIM/ITSM domain in the PD-1 tail and then inhibiting the RAS-ERK and PI3K-NF-κB signaling pathways. Moreover, PD-1 signaling suppresses IFN-γ-activated M1 microglia polarization by reducing the phosphorylation of STAT1 and the secretion of IL-12, while promoting IL-4-activated M2 microglia polarization by increasing STAT6 phosphorylation. **B** Mechanisms of PD-1 signaling in neurons. Activation of the PD-1 pathway dampens neuronal excitation *via* activation of the phosphatase SHP-1/2 and resulting in the downstream modulation of sodium and potassium channels (TREK2 and Kv4.2), as well as $GABA_A$ receptors. Moreover, PD-1 signaling regulates mu-opioid receptor (MOR) function through activation of the phosphatase SHP-1. Red lines ending in a bar represent inhibitory signaling, and black arrows indicate positive signaling. Abbreviations: PD-1, programmed cell death protein 1; PD-L1, PD-1 ligand; SHP, Src homology 2 domain-containing protein tyrosine phosphatase; RAS, a small GTPase encoding *RAS* (retrovirus-associated DNA sequences); ERK, extracellular signal-regulated kinase; PI3K, type I phosphatidylinositol 3-kinase; NF-κB, nuclear factor kappa B; p-STAT 1/6, phosphorylated signal transducer and activator of transcription 1/6; IL, interleukin; IFN-γ, interferon gamma; JAK, Janus kinase; MOR, mu-opioid receptor; TREK2, TWIK-related K^+ channel-2; $GABA_A$R, gamma-aminobutyric acid A receptor; Kv4.2, potassium voltage-gated channel subfamily D member 2.

immunosuppressive expression signatures in non-responders and an enrichment of mitogen-activated protein kinase pathway alterations (PTPN11 and BRAF) in responders [51]. Notably, anti-PD-1 immunotherapy aims to induce a pro-inflammatory environment characterized by increased immune infiltrates into tumors. When this immune checkpoint inhibitor is targeted to treat peripheral tumors, the systemic immune activation may cause central neuroinflammation and associated behavioral and cognitive side-effects. Early clinical studies described some behavioral and cognitive outcomes following anti-PD-1 immunotherapy, including headache, cerebellar ataxia, and transient cognitive dysfunction [52–56]. However, there remains a gap in how these therapies modulate behavioral and cognitive changes. Therefore, pharmacological strategies to cross the BBB and elucidating the mechanisms underlying the immunosuppressive microenvironment, combined with measurement of potential biomarkers that favor anti-PD-1 treatment will improve the efficacy of immunotherapy for clinical brain tumors and metastases, and furthermore, predict adverse CNS events in the treatment of peripheral tumors.

Anti-PD-1 Immunotherapy in Alzheimer's Disease

Alzheimer's disease (AD) is an age-related neurodegenerative disease and the most common cause of dementia [57]. The pathological hallmarks of AD are the extracellular accumulation of β-amyloid (Aβ) plaques which leads to chronic neuroinflammation in the brain [58]. Expression of PD-1 on T cells and PD-L1 on monocytes and macrophages significantly decreases in AD patients and in

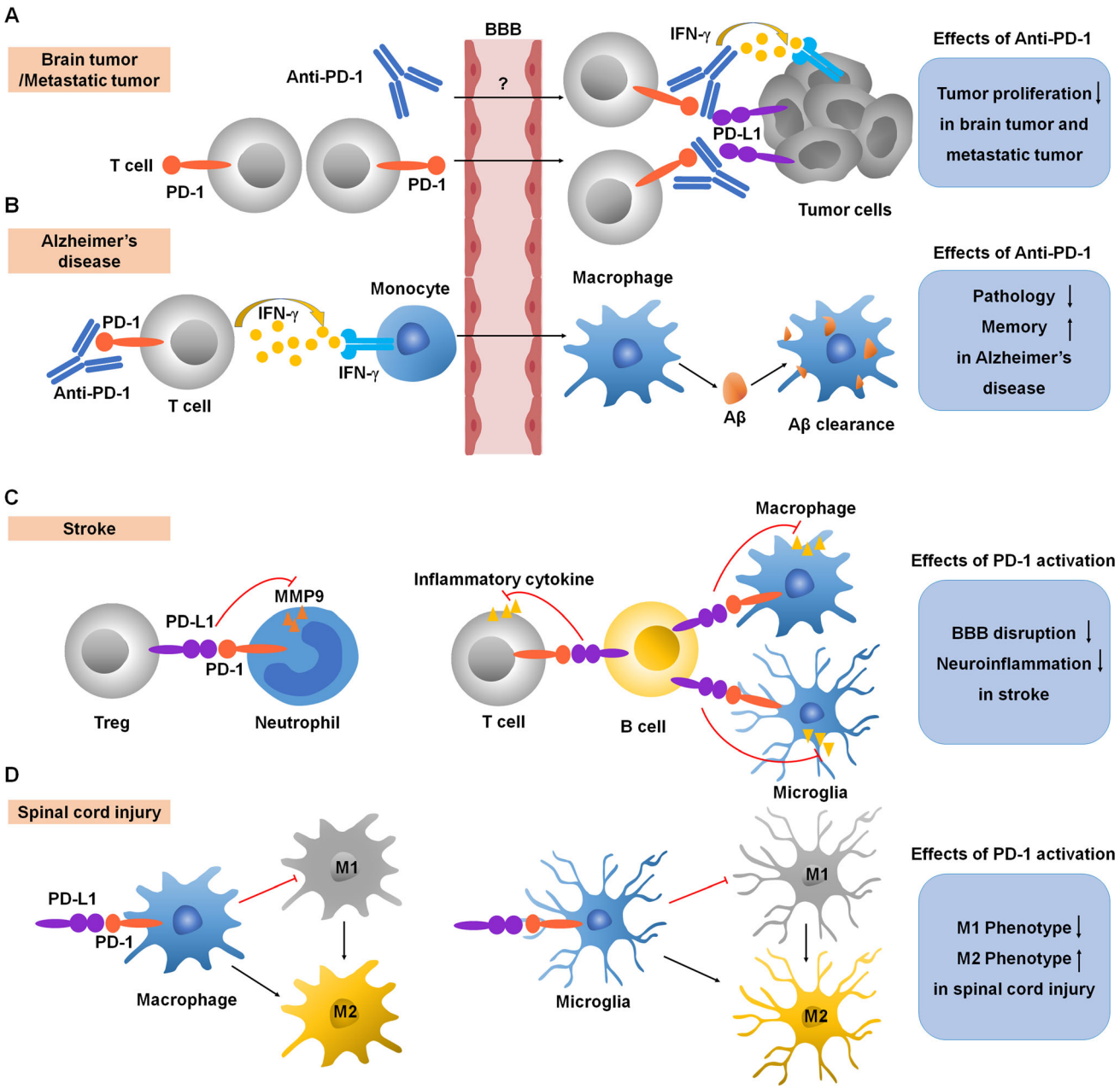

Fig. 4 Immunomodulation by PD-1 in CNS diseases. **A** Anti-PD-1 antibody treatment induces IFN-γ–dependent activity and promotes T cell recruitment to the brain for anti-tumor immunotherapy. **B** Anti-PD-1 antibody treatment evokes a systemic IFN-γ–dependent immune response that enables the mobilization of monocyte-derived macrophages to the brain, thereby reducing pathology and improving memory in Alzheimer's disease. **C** Activation of PD-1 signaling suppresses (1) the release of MMPs from neutrophils, protecting the BBB and (2) the release of inflammatory cytokines from T cell and microglia and macrophages, reducing neuroinflammation in stroke. **D** Activation of PD-1 signaling suppresses microglia and macrophage M1 polarization and promotes M2 polarization in spinal cord injury. Red lines ending in a bar represent inhibitory signaling, and black arrows indicate positive signaling. Abbreviations: PD-1, programmed cell death protein 1; PD-L1, PD-1 ligand; IFN-γ, interferon gamma; BBB, blood-brain barrier; Aβ, β-amyloid; MMP, matrix metallopeptidases.

patients with mild cognitive impairment compared with age- and sex-matched healthy controls, underscoring the importance of PD-1 signaling in AD [59]. Decreased cytokine IL-10 production has been reported in AD patients [60]. Impairment in PD-1 signaling is associated with inhibition of IL-10 production, suggesting that positive PD-1 signaling boosts IL-10 production. IL-10, in turn, has been shown to limit inflammatory responses and ameliorate AD pathology in animal models [61, 62]. Therefore, activation of PD-1 signaling-related

immunoregulatory mechanisms during the progression of AD may help re-establish immune homeostasis. In contrast, in mouse models of AD, the trafficking of blood-borne myeloid cells (monocyte-derived macrophages) to the CNS has also been shown to be neuroprotective. PD-1 blockade evokes a systemic IFN-γ-dependent immune response that enables the mobilization of monocyte-derived macrophages to the brain [63]. In additional studies in rodent AD models, PD-1 blockade reduced cerebral Aβ plaque loads and repeated anti-PD-1 treatment confers long-lasting beneficial effects on AD pathology [64, 65] (Fig. 4b). However, follow-up studies from other groups have shown that inhibition of PD-1 signaling is not sufficient to reduce amyloid pathology in a variety of transgenic AD models [66, 67]. These studies suggest that anti-PD-1 treatments may improve dementia *via* different mechanisms.

PD-1 Signaling in Stroke

Stroke is a devastating CNS condition in which a sudden interruption of blood flow to the brain results in cell death and clinical symptoms such as trouble understanding speech or speaking, paralysis or numbness of the face, arm, or leg, blurred vision, headache, and loss of coordination [68]. Stroke is associated with strong and persistent neuroinflammation. In stroke, the damaged areas of the brain have massive increases in inflammatory factors, activated local microglia, and disruptions of the BBB. There is also major infiltration into the brain of peripheral immune cells, including macrophages, T-cells, and B-cells. PD-1 signaling in T cells and B cells, as well as microphages and microglia, is involved in post-stroke neuroinflammation [69, 70].

Animal models of stroke have shown increased PD-1 expression in activated microglia and macrophages, and that PD-1 deficiency leads to larger brain infarcts and exacerbated neurological deficits. Thus, activation of the PD-1 inhibitory pathway in microglia and macrophages provides a protective effect after stroke (Fig. 4C) [22, 35]. PD-1 expression on B cells leads to inhibition of inflammatory responses in other immune effector cells, and B cells also produce IL-10 and increase the PD-1 expression by T cells, providing neuroprotection against stroke [22, 69, 70].

Another study showed that T regulatory cells mediate the inhibition of neutrophils through PD-1/PD-L1 signaling, and this interaction protects against BBB disruption by suppressing the expression of matrix metalloproteinase-9 (MMP-9) (Fig. 4C) [71]. However, the particular role of PD-L1 in stroke remains controversial. Some studies have shown that PD-L1 exacerbates inflammation in stroke, and treatment with anti-PD-L1 antibodies can control CNS inflammation. Conversely, other studies have demonstrated that PD-L1 significantly attenuates neurological deficits and provides neuroprotection in stroke [72–74]. These opposing results indicate a dual effect of PD-L1/PD-1 signaling in CNS inflammatory conditions. Notably, MMP-9 inhibition is beneficial in the early phase of stroke but detrimental in the late phase of stroke [75]. Time-dependent modulation of neuroinflammation by different MMPs has also been shown in neuropathic pain after nerve injury [76]. Thus, PD-L1/PD-1 signaling in stroke may lead to positive or negative outcomes depending on the different phases and stages of stroke.

PD-1 Signaling in Spinal Cord Injury

Spinal cord injury is a severe CNS condition in which damage to the spinal cord results in paradoxical loss-of-function (e.g., mobility) and gain-of-function (e.g., neuropathic pain) [77–79]. The inflammatory response plays an important role in its pathogenesis and excessive neuroinflammation aggravates the neurological damage after such injury [79, 80]. PD-1 signaling in T cells as well as macrophages and microglia are involved in neuroinflammation after spinal cord injury. One study has shown that the injury impairs T cell cytokine production, and this T cell dysfunction is a result of increased expression of PD-1. Thus, blocking PD-1 signaling can rescue the T cell functionality in spinal cord injury [81]. In addition, PD-1 signaling modulates macrophage and microglial phenotypes after injury. Specifically, PD-1 signaling has been shown to suppress the M1 polarization/phenotype and promote the M2 polarization/phenotype, thereby mitigating neuroinflammation from microglia and macrophages after spinal cord injury (Fig. 4D) [23, 82]. However, the particular molecular mechanism that connects PD-1 signaling to the M1/M2 phenotypic change needs to be further investigated.

PD-1 Signaling in Multiple Sclerosis

Multiple sclerosis is a chronic inflammatory disorder of the brain and spinal cord characterized by focal lymphocytic infiltration leading to the damage of myelin and axons [83]. Expression of PD-1 on T cells and PD-L1 on APCs is increased in multiple sclerosis patients and an impairment of PD-1 inhibitory signaling on T cells as a result of a PD-1 polymorphism is associated with progression of the disease [84, 85]. Preclinical studies of experimental autoimmune encephalomyelitis, an animal model of multiple sclerosis, also showed that genetic deletion of *Pdcd1* or pharmacological blockade of PD-1 enhances the activation and expansion of T-cells and aggravates the pathology in the CNS [86, 87]. Mechanistically, IFN-β has been used to

alleviate multiple sclerosis through up-regulation of PD-L1 and inhibition of CNS neuroinflammation [88]. PD-L1/PD-1 signaling regulates cytokine expression in T cells (IFN-γ and IL-17) and B cells (IL-10) [89, 90]. In addition, IL-12, which is mainly produced by APCs, has also been shown to suppress the development of multiple sclerosis through stimulating IFN-γ production in APCs and enhancing downstream PD-1 signaling [91]. Thus, enhancement of PD-L1/PD-1 inhibitory signaling holds promise as a therapeutic strategy for patients suffering from multiple sclerosis.

Neuromodulation by PD-1 in the PNS and CNS

PD-1 has been studied extensively thus far in non-neuronal cells, and now, PD-1 signaling in neurons is gaining increasing attention. Recent studies have shown that PD-1 is expressed in various neuronal populations including dorsal root ganglion (DRG) sensory neurons, spinal cord neurons, and neurons in specific brain regions, such as the hippocampus [19, 24, 92]. In neurons, PD-1 acts as an inhibitor as it does in immune cells. More specifically, PD-1 expression in neurons affects neuronal excitability, synaptic transmission, and synaptic plasticity (Fig. 5).

Intriguingly, PD-1 is involved in diverse neuronal signaling pathways, four of which are further detailed in this section (Figs. 3B and 5). (1) Activation of PD-1 signaling dampens the excitation of neurons, which occurs *via* the activation of phosphatase SHP-1 and downstream modulation of sodium and potassium channels. PD-1 also affects the characteristics of the neuronal membrane through TWIK-related K^+ channel-2 (TREK2), which is a potassium channel involved in the regulation of the resting membrane potential of sensory neurons [19] (Fig. 5A). SHP-1 has also been shown to dephosphorylate transient receptor potential subtype V1 (TRPV1) in DRG neurons and alleviate inflammatory pain in rats [93]. Furthermore, a recent study has shown that conditioned deletion of SHP-1 in $Na_V1.8^+$ neurons facilitates bone cancer pain [94]. These studies strongly suggest that the phosphatase SHP-1 in nociceptors acts as a pain suppressant. (2) The co-localization of PD-1 with the mu-opioid receptor (MOR) in DRG neurons regulates opioid antinociception [95] (Fig. 5A). (3) PD-1 regulates GABAergic neurotransmission and GABA-mediated analgesia and anesthesia [24] (Fig. 5B). (4) PD-1 in hippocampal CA1 neurons regulates neuronal excitability and synaptic plasticity [92] (Fig. 5C). Due to the important role of PD-1 in neuronal regulation, PD-1 inhibitors may have both beneficial effects (e.g., learning and memory) and detrimental effects (e.g., pain) under different pathological conditions.

PD-1 is broadly expressed by DRG sensory neurons, as well as spinal cord dorsal horn and ventral horn neurons. PD-1 in mouse and human DRG neurons is further transported by axons to their peripheral and central terminals in the skin and spinal cord, respectively [19]. PD-L1 has an analgesic effect that is mediated by PD-1 in naïve mice, as well as in mouse models of inflammatory, neuropathic, and cancer pain [19, 94, 96]. Activation of the PD-L1/PD-1 pathway suppresses action potentials in mouse and human DRG sensory neurons through the modulation of sodium and potassium channels. Furthermore, the TREK2 potassium channel, which regulates the resting membrane potential in C-fiber nociceptors [97], is potentiated by PD-1/PD-L1 signaling in DRG neurons. These modifications of sodium channels and TREK2 potassium channels are regulated by SHP-1, which is activated by PD-L1 in DRG nociceptive neurons *via* phosphorylation (Fig. 5A) [19]. PD-L1 also activates SHP-1 to down-regulate TRPV1 in DRG neurons and delay the development of bone cancer pain in mice [94]. While the PD-L1/PD-1 axis produces acute antinociception through neuromodulation, the delayed effects of this pathway may also depend on immunomodulation. In a mouse model of bone cancer pain, anti-PD-1 treatment with Nivolumab initially increased bone cancer pain through neuronal modulation [98]. In contrast, Nivolumab reduced bone cancer pain in the late phase through modulation of osteoclasts and protection against bone destruction. Thus, anti-PD-1 treatment initially increases cancer pain before reducing it at later time points as a result of both neuromodulation and immunomodulation [98].

PD-1 is co-localized with MOR in DRG sensory neurons and their axons in mouse and human (Fig. 5A). Through interaction with MOR, PD-1 regulates the function of opioid receptors in sensory neurons and plays a crucial role in MOR signaling. PD-1 deficiency or blockade impairs morphine-mediated analgesia in mice and nonhuman primates [95]. Morphine produces antinociception *via* suppression of calcium currents in DRG neurons, inhibition of excitatory synaptic transmission in spinal cord neurons, and induction of outward currents in spinal neurons. But all of these antinociceptive mechanisms are impaired after loss of PD-1 function in *Pd1* (or *Pdcd1*) knockout mice. In addition, loss of PD-1 signaling enhances opioid-induced hyperalgesia and tolerance and potentiates opioid-induced long-term potentiation in the spinal cord [95]. Future studies are warranted to determine how PD-1 interacts with MOR at the molecular level.

Apart from spinal cord neurons, *Pd1* mRNA and PD-1 protein are also widely expressed in neurons of many brain regions, including cortical, thalamic, hypothalamic, and hippocampal neurons. Despite low expression levels, PD-1 in CNS neurons is fully functional. Interestingly, PD-1 is

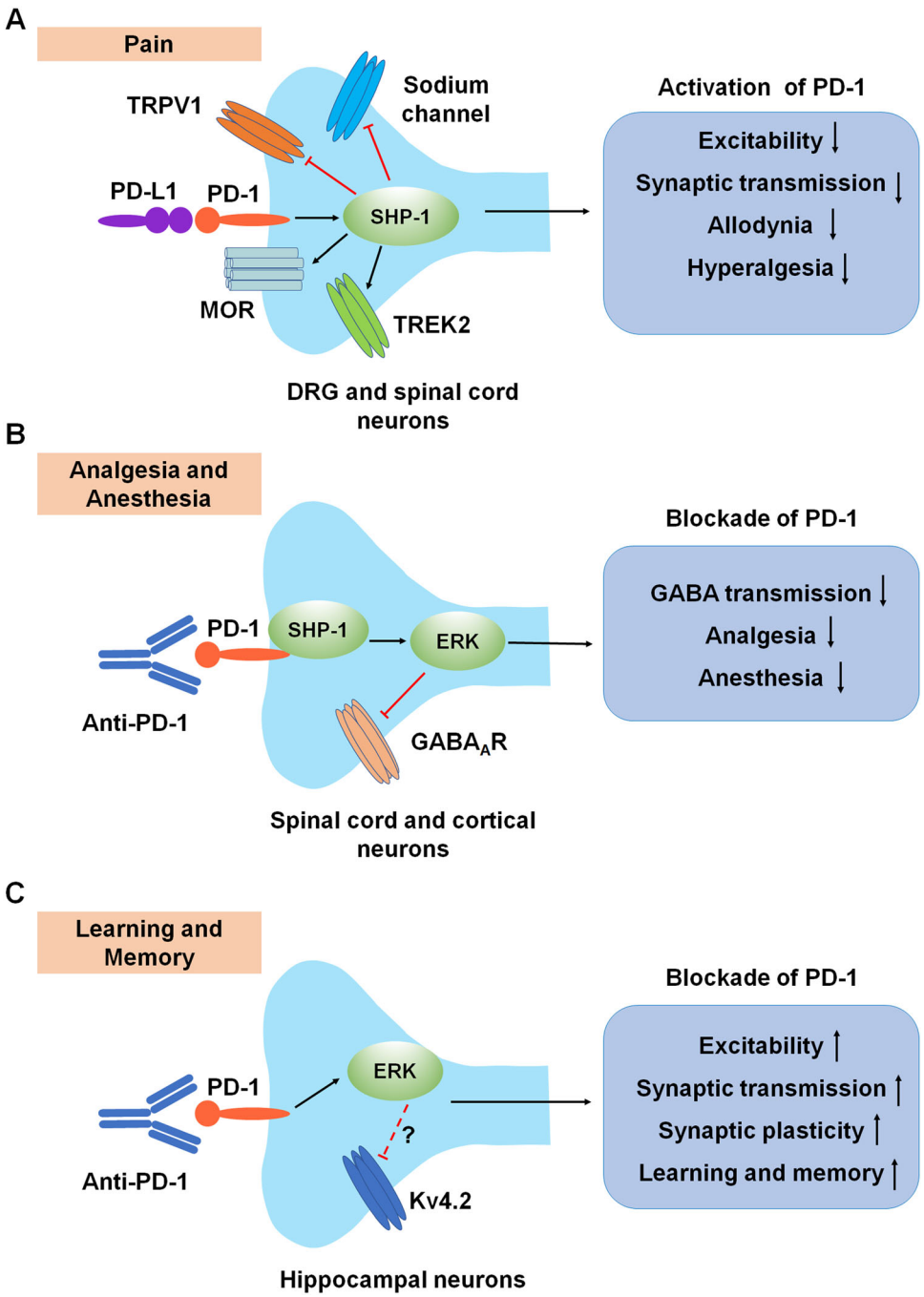

Fig. 5 Neuromodulation by PD-1 in the PNS and CNS. **A** Modulation of pain in primary sensory neurons and spinal dorsal horn neurons. Activation of PD-1 signaling in DRG neurons decreases neuronal excitability and synaptic transmission and inhibits physiological pain and pathological pain (allodynia and hyperalgesia) through modulation of ion channels. **B** Modulation of GABA-mediated analgesia and anesthesia in CNS neurons. **C** Modulation of learning and memory in hippocampal neurons. Anti-PD-1 antibody treatment increases hippocampal neuronal excitability, synaptic transmission, and synaptic plasticity, thereby enhancing learning and memory. Red lines ending in a bar represent inhibitory signaling, and black arrows indicate positive signaling. Abbreviations: PD-1, programmed cell death protein 1; PD-L1, PD-1 ligand; TRPV1, transient receptor potential subtype V1; MOR, mu-opioid receptor; TREK2, TWIK-related K^+ channel-2; DRG, dorsal root ganglion; SHP-1, Src homology 2 domain-containing protein tyrosine phosphatase 1; ERK, extracellular signal-regulated kinase; $GABA_AR$, gamma-aminobutyric acid A receptor; Kv4.2, potassium voltage-gated channel subfamily D member 2.

required for GABAergic neurotransmission, especially the actions of $GABA_ARs$ (Fig. 5B). PD-1 blockade with Nivolumab causes a profound reduction (50%) of GABA-currents across the CNS, including lamina IIo and lamina I in the spinal dorsal horn, S1 sensory cortex, the ventral posterior medial and ventral lateral nuclei of the thalamus, hypothalamus, and the hippocampus. GABAergic neurotransmission is known to mediate analgesia and anesthesia, but strikingly, GABA-mediated analgesia and anesthesia are compromised in $Pd1^{-/-}$ mice [24]. Thus, in CNS neurons, PD-1 is coupled to two inhibitory signaling pathways, mediated by opioid receptors and GABA receptors. Strikingly, PD-1 deficiency or blockade leads to enhanced hippocampal learning and memory [92]. Patch-clamp recording has demonstrated that loss of PD-1 increases neuronal excitability, excitatory synaptic transmission, and synaptic plasticity in hippocampal neurons. Because PD-1 suppresses ERK activation, and ERK phosphorylates the Kv4.2 potassium channel to suppress its activity in hippocampal neurons [99], we postulate that PD-1 signaling

plays an important role as a neuronal inhibitor in learning and memory by regulating the ERK pathway and Kv4.2 potassium channel activity (Fig. 5C). Anti-PD-1 antibodies may potentially serve as a neurotherapy to improve memory function and counteract cognitive decline.

Conclusions and Future Directions

The expression of PD-1 in immune cells, glial cells, and neurons allows for multiple tiers of immunomodulation and neuromodulation in the CNS. PD-1 acts as an inhibitory receptor in various types of cells. Increasing evidence suggests a critical role of PD-1 signaling in CNS resident microglia and peripherally recruited immune cells, as well as neurons under physiological and pathological conditions. Because PD-1 not only regulates immune responses but also neuronal function, PD-1 modulation can exert a range of neuroimmune effects. Thus, the function of PD-1 signaling in the cross-talk between immune cells, glial cells, and neurons in the CNS needs to be further investigated.

To guide rational PD-1-based immunotherapy and neurotherapy in the CNS, several key issues need to be addressed. (1) PD-1-based therapies used for CNS conditions must overcome the obstacles of the BBB. What is the ideal carrier to deliver anti-PD-1 drugs into the brain? (2) The role of PD-1 signaling in restricting local neuroinflammation in the CNS has not been examined. It will be important to determine whether modulation of the PD-1 signaling pathway during CNS injury or neurodegeneration influences the balance between debris clearance, brain repair, and inflammatory damage. (3) PD-1 ligands (e.g., PD-L1 and PD-L2) are expressed by various cell types. What are the specific contributions of these ligands to glial and neuronal functions in CNS disease conditions? (4) Intracellular signaling of neuronal PD-1 must be distinct from that of immune and glial PD-1 due to unique coupling to ion channels. What are the precise molecular mechanisms underlying PD-1 signaling in microglia and neurons in different CNS disease conditions? It is also important to know how PD-1 is coupled to neuron-specific ion channels. (5) To uncover the precise role of PD-1 signaling in immune cells, glial cells, and neurons, conditional-knockout mice will need to be generated to enable specific PD-1 deletions in different cell types and subtypes (e.g., excitatory *versus* inhibitory neurons).

Finally, it is important to point out that PD-1 signaling in the CNS can act like a double-edged sword, producing both beneficial and detrimental effects. A temporary PD-1 signaling blockade, for instance, may cause excitatory effects in microglia and neurons providing beneficial effects under physiological conditions, while a more persistent PD-1 signaling blockade may lead to adverse over-excitatory effects. Thus, the challenge for developing new strategies in using PD-1 signaling therapeutically, will be determining which precise neuronal circuits and cell types need to be tuned for different CNS conditions.

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