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PD-1, gender, and autoimmunity

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Abstract

Programmed death 1 (PD-1) and its ligands (PD-L1 and PD-L2) are responsible for inhibitory T cell signaling that helps mediate the mechanisms of tolerance and immune homeostasis. The PD-1:PD-L signaling pathway has been shown to play an important role in a variety of diseases, including autoimmune conditions, chronic infection, and cancer. Recently, investigators have explored the role of sex hormones in modulating the pathway in autoimmune conditions. Exploring the effects of sex hormones on the PD-1:PD-L pathway could shed light on the gender biased nature of many autoimmune conditions as well as aid in the development of therapeutics targeting the immune system.

Keywords

PD-1; Gender; Sex hormones; Autoimmunity; Systemic lupus erythematosus; Immune system

1. Introduction

Programmed cell death 1 (PD-1) and its ligands, Programmed death ligand 1 (PD-L1) and PD-L2, have an important inhibitory function to play in the regulation of immune homeostasis and in the maintenance of peripheral tolerance through secondary co-stimulatory signaling in activated lymphocytes. PD-1 was discovered upon an increase in its expression following the induction of apoptosis in thymic T cell hybridomas [1]. Encoded by the PDCD1 gene, PD-1 is a 288 amino acid type I transmembrane receptor protein composed of an IgV-like extracellular domain, a transmembrane domain, and a cytoplasmic domain containing an immunoreceptor tyrosine-based inhibitory motif (ITIM) and an immunoreceptor tyrosine-based switch motif (ITSM). PD-1 is a member of the CD28/B7 superfamily of costimulatory molecules that regulates T cell tolerance and is expressed on activated CD4⁺ T cells, CD8⁺ T cells, and antigen presenting cells (APCs) [1–3].

PD-L1 and PD-L2 are both type I transmembrane proteins that have different expression patterns. PD-L1 is found constitutively expressed on murine T cells, B cells, macrophages, dendritic cells (DCs), mesenchymal stem cells, and bone marrow-derived mast cells and is further upregulated in a number of these cell types upon activation [4]. Expression of PD-L1 is also seen in nonhematopoietic cells, such as pancreatic beta cells, vascular endothelial cells, and muscle cells [5–10]. Expression of PD-L2 is restricted to macrophages, DCs, bone marrow-derived mast cells, and peritoneal B1 cells [4,10–13].

Ligation of PD-1 as a secondary signal to T cells in combination with TCR signaling results in a negative/inhibitory co-stimulatory signal [14,15]. The binding of either PD-L1 or PD-L2 to PD-1 results in the recruitment of SH2-domain containing tyrosine phosphatase 1 (SHP-1)

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and SHP-2 to the PD-1 cytoplasmic tail [16]. The subsequent signaling inhibits positive signaling pathways and results in T-cell anergy [17]. PD-1 ligation has been shown to eventually cause a decrease in the induction of cell survival protein Bcl-xL, an inhibition in the expression of transcription factors GATA-3, tbet, and Eomes, and a decrease in the induction of cytokines IL-2 and IFN- γ [18–20]. There is however conflicting data that shows that PD-1:PD-L1 interactions can have positive costimulatory effects [21]. Reverse or bidirectional signaling through PD-L1 and PD-L2 into dendritic cells (DC) following ligation is also possible [14,22,23]. Additionally, recent evidence suggests that PD-L1 acts as a ligand for a second receptor, B7-H1; the ligation of B7-H1 and PD-1 has been shown to inhibit T cell responses [10].

Both PD-1:PD-L signaling and female preponderance in autoimmunity have been well documented. However, only recently has evidence emerged that treatment with sex hormones can have effects on the expression of PD-1. This review will briefly examine the literature on PD-1 and its ligands in a number of diseases as well as discuss the possible connections between the PD-1:PD-L pathway and gender in autoimmunity.

2. PD-1 pathway in tolerance and autoimmunity

PD-1-deficient mice develop spontaneous autoimmune diseases including SLE (nephritis and autoantibodies in C57BL/6 mice and cardiomyopathy in Balb/c mice), indicating an essential function of PD-1 in the mechanisms of tolerance [24–26]. Studies have suggested that PD-1:PD-L1 interaction plays a role in downregulating the activation of T cells [27]. Blockades of PD-1 have been shown to lead to worsening symptoms in murine models of graft-versus-host disease [28] and Experimental Autoimmune Encephomyelitis (EAE) [8]. PD-1 expression on tissue has also been shown to be crucial to promoting peripheral tolerance [13]. Likewise, PD-L1 expression on non-lymphoid cells as well as APCs has been demonstrated to be important in the negative regulation of T cells [29]. However, treatment of BWF1 lupus-prone mice with an anti-PD-1 antibody led to decreases in IFN γ producing CD4⁺ PD-1⁺ T cells and alleviation of autoimmunity, but treatment with an anti-PD-L1 antibody resulted in increases in CD4⁺ PD-1⁺ T cells and worsening nephritis [30].

Knockout of either PD-L1 or PD-1 leads to the development of rapid diabetes with an accompanying increase in insulinitis and T cell production of proinflammatory cytokines in the non-obese diabetes (NOD) mouse model [5,13,31]. Work in the NOD model as well as in an experimental autoimmune encephalomyelitis (EAE) model has shown the importance of PD-1:PD-L1 interactions in controlling autoimmunity, maintaining CD4⁺ T cell tolerance, and inhibiting T cell effector function [7,8,10,13,29,32–36]. PD-1 polymorphisms have been reported to correlate with the incidence of several autoimmune diseases, including SLE, rheumatoid arthritis, diabetes, and multiple sclerosis [37–46]. The PD-1:PD-L1 pathway has also been suggested to be vital in the regulation and function of both the classical CD4⁺ CD25⁺ Tregs [47–49] and the emerging CD8⁺ Tregs [50,51].

3. PD-1 in chronic infection and cancer

Additionally, the role of PD-1 and PD-L1 has also been explored in infectious diseases and cancer. Blockades of PD-1:PD-L1 interaction have been shown to restore antiviral immune function in a model of lymphocytic choriomeningitis (LCMV) [52], and studies have also shown that PD-L1 is upregulated during HIV infection [53]. PD-1 has also been shown to be upregulated on virus-specific T cells during chronic HIV infection [53–56], HCV infection [57], and HBV infection [58]. Blockade of the PD-1:PD-L1 pathway has also been shown to reverse CD4⁺ and CD8⁺ T cell exhaustion in chronic infection [54–57]. Not only viral invaders, but bacteria and parasites, are able to evade immune control due to increases in PD-L1 during persistent infection [17,59–62]. Additionally, several other reports have articulated a role for

the PD-1:PD-L1 pathway in the exhaustion of virus-specific CD8⁺ T cells due to chronic antigen stimulation in infection [52,54–56,63].

The expression of PD-L1 can be found on the surfaces of tumor cells from patients with colon, breast, cervix, ovarian, bladder, and liver cancers to name a few, and many tumor cell lines either express PD-L1 or can be induced to express PD-L1 following stimulation with IFN γ [64–67]. The expression of PD-L1 has been shown to be a good predictor of breast cancer, gastric carcinomas, and esophageal cancers [68–70], and in a study of renal carcinoma patients expression of PD-L1 on tumors was shown to correlate with a marked decrease in survival [71]. Blocking the interaction between PD-1 and PD-L1 has been shown to improve tumor control, inhibit PD-L1 mediated suppression of cytotoxic CD8⁺ T cells, and inhibit T helper cell proliferation and cytokine production—suggesting that PD-1:PD-L1 interactions play a role in inhibiting anti-tumor immunity [64,65,72,73].

4. Autoimmunity, gender, and sex hormones

There has long been a suspected link between autoimmune diseases and sex hormones. Numerous autoimmune diseases, including SLE, rheumatoid arthritis, and systemic sclerosis, have been shown to be more prevalent in women [74]. In the case of many of these diseases, sex hormones have been implicated to play an important role. In SLE, both estrogen and prolactin have been shown to be immunomodulators that can allow autoreactive B cells to evade tolerance mechanisms and proliferate [75–77], while treatment with testosterone [74, 78], estrogen receptor antagonists [79–81], or inhibitors of prolactin secretion [82,83] have been shown to alleviate disease in murine models of SLE as well as in certain groups of SLE patients. Additionally, X chromosome genes have been demonstrated to play possible roles in systemic sclerosis, autoimmune thyroid disease, and SLE [74].

5. PD-1 and sex hormones

A role for sex hormones in the modulation of PD-1 has recently emerged. The Offner group has extensively examined the role of Tregs and the PD-1 pathway in the estrogen mediated immunomodulation of EAE mice. They have reported that 17 β -Estradiol (E2) treatment in EAE mice alleviates disease and leads to increases in the CD4⁺CD25⁺ Treg compartment, FoxP3 expression, and expression of PD-1 on Tregs, macrophages, B cells, and dendritic cells [84–86]. Interestingly, Tregs were shown to be necessary for E2-mediated attenuation of disease [87]. After treatment with E2, EAE mice showed increases in the intracellular expression of PD-1 in Tregs in a dose dependent manner [87,88] with the frequency of Tregs increasing following administration of pregnancy levels of E2 [89]. Immunized PD-1 knockout mice had a delayed onset of disease but not complete protection from EAE following administration of E2 [87]. Prior to treatment, PD-1 KO mice had normal levels of FoxP3 expression, but had Tregs that did not show detectable suppressive capacity. E2 treatment only slightly increased PD-1 KO Tregs suppressive capacity and did so without increasing the expression of FoxP3 [88].

The estrogen sensitivity of PD-1 expression was further supported by experiments showing estrogen receptor alpha (Esr1) knock out (ERKO) mice had a significant decrease in PD-1 expression in FoxP3⁺ cells. There were, however, only minor differences in FoxP3 expression or extracellular PD-1 expression between wild type, ERKO, and Esr2 KO (BERKO) mice [88]. These results suggested that PD-1 was essential for suppression even with normal FoxP3 expression.

Immunized WT type mice had decreases in the level of IL-17 following treatment with E2, but PD-1 KO mice actually saw a slight increase in IL-17 following E2 treatment [88]. These data suggested that the presence of PD-1 is important in the IL-17 response to estrogen. Treatment

of EAE mice with G-1, an agonist that selectively activates the membrane bound estrogen receptor G protein receptor 30 (GPR30), has also been shown to alleviate symptoms of EAE in mice [89]. Concomitant with GPR30 mediated alleviation of disease was an increase in the expression of PD-1 on CD4⁺ FoxP3⁺ Tregs. These PD-1⁺ Tregs were shown to have an increased suppressive capacity suggesting that signaling through GPR30 was enough to engage the T regulatory cells to greater suppressive capacity [89].

Most interestingly, studies indicate that E2 mediates its effects on the Treg compartment indirectly through antigen presenting cells and nonlymphocytic cells [90]. Co-cultures of T cells from either E2 treated or untreated EAE mice with E2 treated macrophages showed marked reduction of inflammatory cytokines IL-12 and IFN γ and increases in IL-10. However, co-cultures of either treated or untreated T cells with untreated macrophages showed the opposite pattern with increases in IL-12 and IFN γ and decreases in IL-10 [86]. Bone marrow-derived dendritic cells (BM-DC) treated with E2 demonstrated the ability to suppress responder CD4⁺CD25⁻ T cells either in the presence or absence of CD4⁺ Tregs in culture [86].

E2 treatment of immunized GPR30 KO mice only partially abrogated E2-mediated suppression of disease [89]. Along with data indicating that E2 is able to partially increase Treg suppression in PD-1 KO mice, these findings indicate that E2's role in promoting alleviation of EAE is complex and includes mechanisms outside of PD-1 increases in the Treg compartment [86].

These results taken in tandem show an important link between PD-1 and E2 mediated protection from EAE. E2 treatment seems to alleviate EAE symptoms by increasing expression of PD-1 in the Treg compartment. This increase appears to be mediated by estrogen receptor alpha as well as in part by the membrane estrogen receptor GPR30. The E2 mechanism of action might also be in part mediated by the effect of E2 on DCs and APCs, which can then go on to program T cells to a regulatory phenotype through PD-1 signaling. These links between estrogen, PD-1, and the regulatory activity of Tregs suggests that estrogen's role in autoimmunity might be complex and multitudinous.

6. Concluding remarks

Interactions between PD-1 and its ligands play a variety of roles in T cell activation and the maintenance of immune homeostasis, and have been shown to have an important role in autoimmune diseases as well as in cancer and chronic viral infections. Recent research suggests that the expression and function of PD-1 is responsive to treatment by hormones and that these hormone mediated effects on the PD-1 signaling pathway can play an important role in modulating autoimmunity. Understanding the effects of sex hormones on the PD-1:PD-L pathway shows the promise of delineating gender bias in autoimmune diseases. Therapeutic agonists and antagonists targeting these pathways could have many beneficial effects in treating a host of diseases, including autoimmune conditions and cancer.

Take-home messages

- The PD-1:PD-L pathway mediates inhibitory T cell signaling and has an important role to play in autoimmune diseases, cancer, and viral infections.
- Blockades of PD-1 in Experimental autoimmune encephalomyelitis and murine graft vs. host disease have been shown to worsen disease and PD-1 deficient mice develop autoimmunity.
- Autoimmune diseases are sex-biased with women more likely than men to be susceptible to diseases such as systemic lupus erythematosus, rheumatoid arthritis, and systemic sclerosis.

- Treatment of EAE mice with 17 β -estradiol (E2) has been shown to alleviate disease concomitant with increases in PD-1 expression in the Treg compartment.
- Preliminary studies suggest that studying the role of sex hormones on PD-1 expression and PD-1:PD-L signaling could yield information that could further help in understanding the sex biased nature of numerous autoimmune diseases.

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