Review

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Human cancer immunotherapy with antibodies to the PD-1 and PD-L1 pathway

Kim C. Ohaegbulam[1*], Amer Assal[2*], Eszter Lazar-Molnar[3], Yu Yao[4], and Xingxing Zang[1,2]

[1] Department of Microbiology and Immunology, Albert Einstein College of Medicine, New York, NY 10461, USA
[2] Department of Oncology, Montefiore Medical Center, New York, NY 10467, USA
[3] Department of Pathology, University of Utah School of Medicine, Salt Lake City, UT 84132, USA
[4] Department of Neurosurgery, Huashan Hospital, Fudan University, Shanghai 200040, China

The programmed death 1 (PD-1) receptor and its ligands programmed death ligand 1 (PD-L1) and PD-L2, members of the CD28 and B7 families, play critical roles in T cell coinhibition and exhaustion. Overexpression of PD-L1 and PD-1 on tumor cells and tumor-infiltrating lymphocytes, respectively, correlates with poor disease outcome in some human cancers. Monoclonal antibodies (mAbs) blockading the PD-1/PD-L1 pathway have been developed for cancer immunotherapy via enhancing T cell functions. Clinical trials with mAbs to PD-1 and PD-L1 have shown impressive response rates in patients, particularly for melanoma, non-small-cell lung cancer (NSCLC), renal cell carcinoma (RCC), and bladder cancer. Further studies are needed to dissect the mechanisms of variable response rate, to identify biomarkers for clinical response, to develop small-molecule inhibitors, and to combine these treatments with other therapies.

Expression of PD-1 and its ligands

The PD-1 (CD279) (see Glossary) receptor can be detected at the cell surface of T cells during thymic development and in the periphery of several types of hematopoietic cell following T cell receptor (TCR) signaling and cytokine stimulation. PD-1 is expressed on $CD4^{-}CD8^{-}$ thymocytes and inducibly expressed on peripheral $CD4^{+}$ and $CD8^{+}$ T cells, B cells, monocytes, natural killer (NK) T cells, and some dendritic cells (DCs) [1,2]. Persistent expression of PD-1 on T cells induces T cell exhaustion [3]. Exhausted CD8 T cells lose their effector function, evidenced by their inability to secrete cytolytic molecules such as perforin and their failure to secrete proinflammatory cytokines, such as IL-2, interferon gamma (IFN-γ), and tumor necrosis factor alpha (TNF-α) [4,5].

$CD4^{+}Foxp3^{+}$ regulatory T cells (Tregs), a highly immunosuppressive subset of $CD4^{+}$ T cells that is critical in maintaining tolerance and attenuating immune responses, express cell-surface PD-1, which contributes to their development, maintenance, and functional response [6]. Ligand binding to the PD-1 receptor on Tregs in the presence of CD3 and transforming growth factor beta (TGF-β) leads to an increase in the *de novo* conversion of naive $CD4^{+}$ T cells to Tregs. This induction generates heightened suppressive function and maintenance of Foxp3 expression through inhibition of Akt–mammalian target of rapamycin (mTOR) signaling and increased phosphatase and tensin homolog (PTEN) activity [7,8]. This indicates that PD-1 pathway stimulation results not only in a reduction in effector T cell function, but also an increase in immunosuppressive Treg function. This allows proper control of immune homeostasis and creates a high threshold for T cell activation.

Although PD-1 has best been characterized in T cells, its function in other cell subsets have also become apparent. The regulation of PD-1 expression is tightly controlled during B cell differentiation. Levels are undetectable in pro-B cells, an early precursor in B cell development, and increase as B cell differentiation [9]. Additionally, surface levels of PD-1 can be greatly enhanced in mature B cells following stimulation with Toll-like receptor 9 (TLR9) agonists. Blockade of PD-1 on B cells has been shown to increase antigen-specific antibody responses,

Glossary

Cancer immunotherapy: treatments that use the host immune system to inhibit cancer.
Monoclonal antibody (mAb): antibodies generated by immune cells derived from a single parent cell.
Programmed death 1 (PD-1): a 288-amino acid cell-surface molecule, encoded in humans by the *PDCD1* gene, that functions to negatively regulate immune responses.
Programmed death ligand 1 (PD-L1): a 40-kDa type 1 transmembrane protein, encoded in humans by the *CD274* gene, that suppresses the immune system in cancer, pregnancy, tissue allografts, and autoimmune diseases.
T cell: a type of lymphocyte that has an important role in cell-mediated immunity, distinguished by its T cell receptor on the cell surface; referred to as T cells because they mature in the thymus.
T cell coinhibition: a signal required for inhibition of activated T cells in the presence of T cell receptor signal.

Corresponding author: Zang, X. (xing-xing.zang@einstein.yu.edu).
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*These authors contributed equally.

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suggesting that PD-1 plays a role in inhibiting B cell clonal responses [10].

PD-1 has two binding ligands, PD-L1 (B7-H1, CD274) [11,12] and PD-L2 (B7-DC, CD273) [13,14], with PD-L1 being the most prominent in regulation. PD-L1 is inducibly expressed on both hematopoietic cells and non-hematopoietic cells following cell-specific stimulation. Cytokines such as IFN-γ and TNF-α upregulate the expression of PD-L1 on T cells, B cells, endothelial cells, and epithelial cells, furthering its role in the maintenance of peripheral tolerance [1]. Data also link genetic changes seen in cancer cells to the induction of PD-L1, although this can vary by cancer type. PTEN dysfunction in human glioma cells induces Akt activation and subsequently PD-L1 expression, while human melanoma cells show no association between PTEN or Akt and PD-L1 induction [15,16]. Recent data show that PD-L1 binds to B7-1 (CD80) in addition to PD-1 [17]. While PD-L1 expression is induced on a wide array of both hematopoietic and non-hematopoietic cells, PD-L2 expression is restricted to inducible expression on DCs, macrophages, mast cells, and some B cells in response to IL-4 and IFN. The affinity of PD-L2 for PD-1 is three times greater than that of PD-L1, which indicates competition between the two ligands. Recent data confirm a second cognate receptor for PD-L2, repulsive guidance molecule B (RGMb) [18]. Despite recent research efforts surrounding PD-L2, little is known regarding the transcriptional regulation of the ligand.

Structures of PD-1 and its ligands

Structurally, PD-1 is a type I transmembrane receptor and belongs to the Ig superfamily (IgSF). Although it is functionally related to the costimulatory/coinhibitory receptors CD28, cytotoxic T lymphocyte-associated protein 4 (CTLA-4) and inducible T cell costimulator (ICOS), PD-1 has important structural and functional differences. Other receptors in the CD28 family are disulfide-linked dimers; however, structural and cell-surface studies demonstrated that PD-1 is a monomeric glycoprotein [19]. The crystal structure of the extracellular region of mouse PD-1 shows the presence of a typical Ig variable domain (IgV) comprising front sheets (A'GFCC'C") and back sheets (ABED) (Figure 1) stabilized by a disulfide bond linking the F and B strands [19]. This IgV domain is linked to transmembrane and cytoplasmic domains through a 20-amino acid stalk region. In contrast to other CD28 family receptors, the absence of an extracellular cysteine residue in the stalk region prevents PD-1 from covalent dimer formation.

Human and mouse PD-1 share around 60% overall identity at the protein level, which increases to 75% for the residues forming the IgV domain. It is unsurprising, therefore, that crystallographic [Protein Data Bank (PDB) code 3RRQ] and NMR structures [20] show a high degree of similarity between mouse and human PD-1. Overlay of the crystal structures of mouse and human PD-1 shows very similar arrangements (Figure 1). One notable difference between human and mouse PD-1 is the lack of the C" strand at the edge of the front GFCC' sheet in human PD-1, as shown by the NMR data [20]. This region instead presents as a highly flexible loop, consistent with the poor

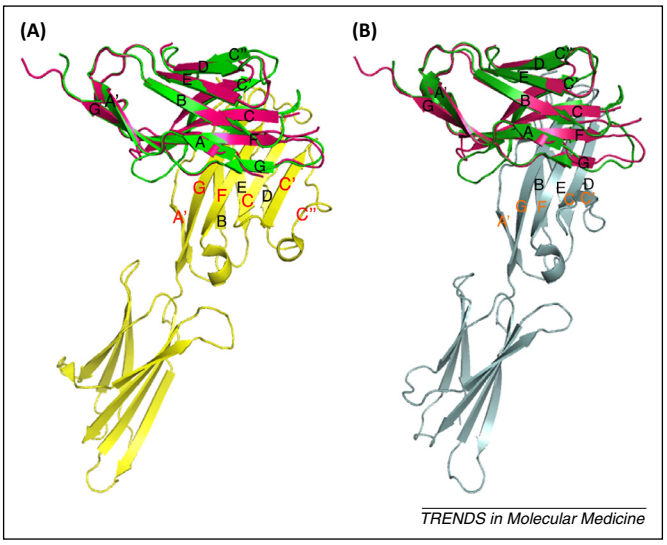

Figure 1. Crystal structures of programmed death 1 (PD-1) receptor/programmed death ligand 1 (PD-L1) and PD-1/PD-L2 complexes. **(A)** Overlay of the crystal structures of human PD-1 [Protein Data Bank (PDB) code 3RRQ] and the mouse PD-1/human PD-L1 complex (3BIK). **(B)** Overlay of human PD-1 with the mouse PD-1/PD-L2 complex (3BP5). Pink, human PD-1; green, mouse PD-1; yellow, human PD-L1; grey, mouse PD-L2.

electron density observed for that region in the crystallographic dataset, indicating a disordered arrangement (PBD code 3RRQ).

Another unique structural feature of PD-1 is that it lacks a consensus complementarity-determining region 3 (CDR3)-like conserved ligand-binding motif. The ligand-binding site comprises a hydrophobic patch on the front face contributed by multiple residues from several strands [19]. Crystal structures available for complexes of mouse PD-1 and human PD-L1 [21] and mouse PD-1 with mouse PD-L2 [22] show similar overall molecular architecture for these inhibitory complexes. Both PD-1 and its ligands interact with their respective surface residues distributed over their front beta sheets (front-to-front binding). By contrast, the FG loop of PD-1, which corresponds to the CDR3 variable region of the Ig structure, makes little or no contact with PD-L1 or PD-L2 (Figure 1). The crystal structures of the PD-1/PD-L complexes reveal that PD-1 binds its ligands with 1:1 stoichiometry and forms monomeric complexes. This indicates a distinct ligand-binding mode and signaling mechanism that differs from other coinhibitory receptor/ligand interactions such as CTLA-4/B7, where oligomerization plays an important role in signaling. Although crystal structures for the human PD-1/PD-L1 and PD-1/PD-L2 complexes remain to be solved, the overall similarity of the mouse and human PD-1 structures suggests that mouse and human PD-1 are likely to form similar complexes. Overlay of human PD-1 with the mouse complexes shows that human PD-1 may bind to its ligands in the same way as mouse PD-1; however, a recent study using NMR with binding data and mathematical modeling suggests that PD-1 may be engaged by its two ligands differently [20].

Importantly, the available crystal structures of PD-1 and the PD-1/PD-L1 and PD-1/PD-L2 complexes allow not only mapping of the ligand-binding sites and mAb blocking epitopes, but also the design of small-molecule inhibitors.