Structures of PD-1 with its ligands: Sideways and dancing cheek to cheek

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T cell activation requires a TCR-mediated signal, but the strength, course, and duration are directed by costimulatory molecules and cytokines from the antigen-presenting cell (APC). An unexpected finding was that some molecular pairs attenuate the strength of the TCR signal, a process termed coinhibition (reviewed in refs. 1–3). The threshold for the initiation of an immune response is set very high, with a requirement for both antigen recognition and costimulatory signals from innate immune recognition of "danger" signals. Paradoxically, T cell activation also induces expression of coinhibitory receptors such as programmed death-1 (PD-1). Cytokines produced after T cell activation such as INF-γ and IL-4 up-regulate PD-1 ligands, establishing a feedback loop that attenuates immune responses and limits the extent of immune-mediated tissue damage unless overridden by strong costimulatory signals. PD-1 is a CD28 family member expressed on activated T cells, B cells, and myeloid cells. In proximity to the TCR signaling complex, PD-1 delivers a coinhibitory signal upon binding to either of its two ligands, PD-L1 or PD-L2. Engagement of ligand results in tyrosine phosphorylation of the PD-1 cytoplasmic domain and recruitment of phosphatases, particularly SHP2 (Fig. 1). This results in dephosphorylation of TCR proximal signaling molecules including ZAP70, PKCθ, and CD3ζ, leading to attenuation of the TCR/CD28 signal (4). The role of the PD-1 pathway in peripheral T cell tolerance and its role in immune evasion by tumors and chronic infections make the PD-1 pathway a promising therapeutic target. Two recent papers have determined the structures of the PD-1/PD-L1 (5) and PD-1/PD-L2 complexes [see Lazar-Molnar *et al.* (6) in this issue of PNAS].

PD-L2 (B7-DC; CD273) is inducibly expressed on dendritic cells and macrophages, whereas PD-L1 (B7-H1; CD274) is broadly expressed on both professional and nonprofessional APCs as well as a wide variety of nonhematopoietic cell types (1–3). The PD-1 pathway is important for the maintenance of peripheral T cell tolerance. Disruption of the *Pdcd1* gene can accelerate autoimmune diseases in mice including a lupus-like disorder in *lpr* mice or diabetes in nonobese diabetic (NOD) mice. Expression of PD-L1 on nonhematopoietic cells inhibits pathogenic self-reactive T cells in NOD mice.

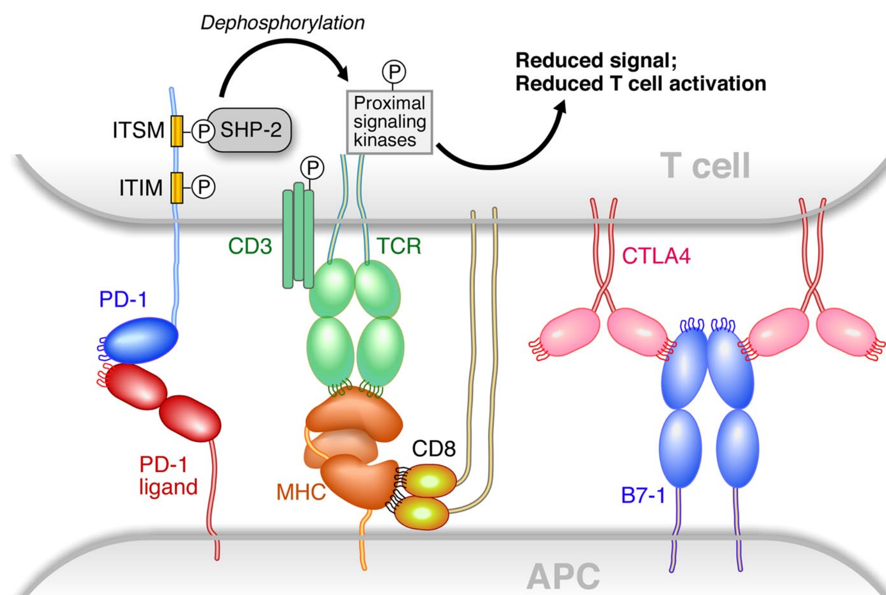

Fig. 1. Structures in the B7/CD28 family. Structures are modeled on the crystal determinations. Loops have been added to one end of the IgV domains to emphasize the orientation of the CDR-like loops and their interaction with ligand or lack thereof.

Chronic infections and tumors have exploited the PD-1 pathway to evade eradication by the immune system. In acute infections, PD-1 is up-regulated upon T cell activation and declines with resolution of the infection and establishment of memory (7). Studies in the lymphocytic choriomeningitis virus (LCMV) model of chronic viral infection show that PD-1 expression on virus-specific T cells remains high, and these T cells become "exhausted," with progressive loss of effector functions and proliferative capacity. Antibody blockade of the PD-1 pathway enhanced virus-specific CD8 T cell responses *in vivo*, resulting in increased proliferation, cytokine production, cytolytic activity, and a reduction in viral load. Exhausted antigen-specific CD8 T cells have been observed in chronic human infections with HIV, HBV, and HCV as well as SIV infection of monkeys. Recent work has shown that these exhausted viral-specific T cells express high levels of PD-1 and that blockade of the PD-1 pathway can enhance *in vitro* T cell responses (reviewed in ref. 2).

PD-L1 is expressed on a wide variety of tumors and is a component of the immunosuppressive milieu (8, 9). Studies in animal models demonstrate that PD-L1 on tumors inhibits T cell activation and lysis of tumor cells and in some cases leads to increased death of tumor-specific T cells. PD-L1 expression on human tumors strongly correlates with unfavorable prognosis in kidney, ovarian, and bladder cancer (10). A number of groups have developed mAbs against PD-1 or its ligands for therapeutic use. Trials with tumors and chronic viral diseases are beginning and the results are eagerly awaited.

The two recent papers describing the structures of PD-1 with its ligands may guide the development of improved therapeutics. The PD-1 ectodomain contains a single IgV domain typical of the CD28 family, whereas PD-L1 and PD-L2 are composed of IgV and IgC domains typical of the B7 family. The structures show a 1:1 receptor/ligand stoichiometry, with interaction primarily between the faces of the IgV domains. PD-1, PD-L1, and

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PD-L2 are monomers in the crystal and on the cell surface unlike B7–1, CD28, and CTLA4, which are noncovalent and covalent dimers, respectively (11, 12). An IgV domain is composed of ≈120 aa organized into nine parallel β-strands (ABCC′C″DEFG) with loops connecting the strands. The B and F strands are connected by the canonical Ig domain disulfide bond, resulting in a two-layered sandwich structure with two faces. Antibodies and T cell receptors generate sequence diversity in the BC, C′C″ and FG loops and use these complementarity-determining regions (CDR) to specifically bind antigen. In contrast, the large Ig superfamily of genes may bind ligand using any face of the IgV domain or the loops. CTLA4 uses primarily the MYPPPY motif in the CDR3 region (FG loop) as well as the CDR1 loop to bind to the face of B7–1 or B7–2 (12, 13). In contrast, PD-1 uses the front β-face (GFCC′ strands and CC′, CC″, and FG loops) to bind to the front β-face of PD-L1 (GFCC') or PD-L2 (AGFC strands and FG loop). This buries a large surface area (≈1,900 Å^2 versus 1,200 for B7/CTLA4). Six amino acids of the C, F, and G strands of PD-1 form a concave, hydrophobic core that interacts with the F and G strands as well as the FG loop of PD-L2. Eight of 14 aa involved in binding to PD-1 are identical or highly conserved between PD-L1 and PD-L2. Trp-110 in the middle of the G strand of PD-L2 is an Ala in PD-L1, and mutational analysis suggests that this accounts for much of the 3-fold-higher affinity of PD-L2 for PD-1 (6, 14). Binding face to face establishes an acute angle between PD-1 and PD-L1 or PD-L2, as opposed to the right angle between CTLA4 and B7–1. This acute angle shortens the distance between the distal ends of the PD-1 and PD-L1 or PD-L2 molecules (76 Å as opposed to the 100 Å of B7–1/CTLA4). This likely explains the longer segments connecting the Ig domain to the membrane (20 and 11 aa for PD-1 and PD-L2 as opposed to 6 and 9 for CTLA4 and B7–1). Both molecular pairs are in the immunologic synapse and should span ≈140 Å, compatible with the dimensions of TCR/MHC (15). PD-1 and PD-L1 can be expressed on the same cell, and an open question is whether there is sufficient rotational freedom in the connecting segments to allow PD-1 to bind to an adjacent PD-1 ligand molecule on the same cell surface.

A major difference between PD-L1 and PD-L2 is a 14-aa gap in the IgV domain of PD-L2. This deletes the C″ strand of PD-L2, and the shortened C′ strand lies above and at a right angle on the lower portion of the GFC face. In contrast, the C′ and C″ strands of PD-L1 lie in classic parallel orientation with the GFC strands. These differences leave the upper portion of the GFC faces of PD-L1 and PD-L2 very similar and this is where PD-1 binds. The lower portion of the GFC face is very different. A surprising recent result is that B7–1 is a ligand for PD-L1 with an affinity between that of B7–1 for CD28 and CTLA4 (14). The binding site was mapped to the IgV domains of B7–1 and PD-L1, and binding inhibits T cell activation. Because PD-L2 did not bind to B7–1, regions on the IgV domain that differ between PD-L1 and PD-L2 such as the lower portion of the GFC face are attractive candidates for the binding of B7–1. A cocrystal structure will be informative.

With the IgV/IgC structures of B7–1, PD-L1, and PD-L2 now determined, the defining structure of the B7 family appears to be a slender rod as opposed to the angled structure of Ig superfamily members like CD4. A linear strand of 5–6 aa connects the IgV and IgC domains and the amino acids making contacts between the bottom of the IgV and the top of the IgC domains are highly conserved. The structures of the complete ectodomains of B7–1 and PD-L1 alone and with ligand have been determined. Although the complete structure of PD-L2 with ligand was determined, the structure of free PD-L2 is only of the IgV domain. B7–1 is a rigid rod with a very similar structure alone or bound to CTLA4 (11, 12). In contrast, the IgV and IgC domains of PD-L1 are in a straight line when complexed with PD-1 but diverge 38° from straight without PD-1 (5). This flexibility between the two domains suggests PD-L1 may accommodate to the orientation of receptor during binding (5). An unanswered question is how binding of ligand transduces a signal. In both PD-L1 and PD-L2, the CC′ loop moves 2 Å to contact the G strand of PD-1. In PD-1, the FG loop moves 1.3 Å; however, neither movement was considered sufficient to transduce a signal.

When compared with the protein structure database, the IgV domains of the PD-1/PD-L1 structure have the greatest similarity to the αβ structure of antigen receptors and of CD8 dimers (5). Intriguingly, the face-to-face binding allows the CDR-like loops of both PD-1 and PD-L1 or PD-L2 to point in the same sideways direction, raising the possibility that the combined structure might generate a binding site for an additional, unidentified ligand. By analogy, CD8 monomers do not bind MHC class I but CD8 αα or αβ dimers bind face to face and use their combined six CDR-like loops to bind the α3 domain of MHC class I (16) (Fig. 1).

A number of mutations that reduce ligand binding have been identified. Although many correspond to amino acids that make contact, some turn out to be distant from the binding site and presumably have long-distance effects upon the structure (5, 6). The PD-1 mutant A99L had a 3-fold-higher affinity for ligand and may have enhanced efficacy as a therapeutic agent. These structures give insight into function, explain higher-affinity forms of the molecules, guide further affinity improvement, and make interesting new biophysical predictions to explore.

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