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Biochemical Signaling of PD-1 on T Cells and Its Functional Implications

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Abstract

Maintenance of peripheral tolerance is essential for homeostasis of the immune system. While central tolerance mechanisms result in deletion of the majority of self-reactive T cells, T lymphocytes specific for self-antigens also escape this process and circulate in the periphery. To control the development of autoimmunity, multiple mechanisms of peripheral tolerance have evolved, including T cell anergy, deletion and suppression by regulatory T cells (Treg). The pathway consisting of the PD-1 receptor (CD279) and its ligands PD-L1 (B7-H1, CD274) and PD-L2 (B7-DC; CD273) plays a vital role in the induction and maintenance of peripheral tolerance. This pathway also regulates the balance between stimulatory and inhibitory signals needed for effective immunity and maintenance of T cell homeostasis. In contrast to this important beneficial role in maintaining T cell homeostasis, PD-1 mediates potent inhibitory signals that prevent the expansion and function of T effector cells and have detrimental effects on anti-viral and anti-tumor immunity. In spite of the compelling studies on the significant functional role of PD-1 in mediating inhibition of activated T cells, little is known about how PD-1 blocks T cell activation. Here, we will provide a brief overview of the signaling events that are regulated by PD-1 triggering and we will discuss their implications on cell intrinsic and extrinsic mechanisms that determine the fate and function of T effector cells.

Introduction

PD-1 is a 288 amino acid protein, which is induced on T cells upon activation via the T cell receptor and via cytokine receptors^{1–4}. PD-1 was initially identified as a molecule responsible for induction of cell death⁵. In contrast to its robust induced expression in activated mature T cells, PD-1 is expressed at low levels on CD4⁺ CD8⁺ double negative $\alpha\beta$ and $\gamma\delta$ T cells in the thymus⁶, suggesting that PD-1 has a dominant role in regulating peripheral and not central tolerance. PD-1 expression is also induced upon activation of natural killer T (NKT) cells, B cells, monocytes and certain subsets of dendritic cell (DC)^{4, 7–9}. The ligands for PD-1, PD-L1 (also known as B7-H1) and PD-L2 (also known as B7-DC) have distinct expression patterns^{10–13}. PD-L1 is constitutively expressed in low levels on APCs (DCs, macrophages, and B cells) and is further upregulated upon their activation. PD-L1 is also induced on activated T cells^{1, 14}. In addition, PD-L1 is expressed

on a wide variety of nonhematopoietic cell types, including vascular endothelial cells, pancreatic islet cells, and sites of immune privilege including the placenta, testes, and eye. In contrast, expression of PD-L2 is induced primarily on DCs and macrophages upon activation^{14, 15}. The identification of B7-1 as a ligand for PD-L1¹⁶ also indicates that the physiologic role of this pathway in regulating T cell tolerance in specific microenvironments depends not only on the expression of PD-L1/2 but also on the selective and differential expression of B7-1.

Due to the cell-specific and tissue-specific distribution of PD-1 ligands, PD-1 mediates its effects during different phases of T cell activation. Specifically, PD-1 could exert its function during the initial phase of activation of autoreactive T cells by attenuating self-reactive T cells during presentation of self-antigen by dendritic cells (DCs)^{17, 18}. A major role of PD-1, however, is to inhibit the functions of self-reactive and inflammatory effector T cells against non-hematopoietic tissues and mediates tissue tolerance to protect against immune-mediated tissue damage^{13, 19}. The activation-induced expression of PD-1 suggests that PD-1-dependent inhibition functions after the initiation and rather in later phases of the immune response, which support sustained activation and T cell expansion²⁰. Consistent with this property of PD-1 is the finding that when naïve TCR-transgenic T cells expressing the DO11.10 TCR from the PD-1 deficient and from WT DO11.10 transgenic mice were stimulated with PD-L1⁺ APC *in vitro*, PD-1 deficient T cells displayed higher numbers of cells with more than three divisions but not higher numbers of cells with one to two divisions, compared with their WT type counterparts²¹. The observation that implementation of PD-1 function requires prior T cell activation is further supported by the fact that transcription of PD-1 itself requires nuclear translocation of NFAT and binding for NFATc1 (NFAT2) to the promoter of the *PDCDI*, the gene encoding PD-1, inducing its transcription²². Together these findings suggest that PD-1 expression is induced after T cell activation, and subsequently, T cells expressing PD-1 become sensitive to PD-1-mediated inhibition through ligation by PD-L1/PD-L2. In addition to mediating T cell-intrinsic inhibitory effects by mediating signals to PD-1 positive activated T cells, PD-1 inhibits T cell responses by promoting the induction and maintenance of inducible Tregs (iTregs)²³.

The role of PD-1 in the regulation of peripheral tolerance was first indicated by the finding that aged PD-1 deficient mice develop lupus-like glomerulonephritis and arthritis on the C57BL/6 mouse strain²⁴. This condition highly resembles systemic lupus erythematosus (SLE), a prototype of human systemic autoimmune diseases, which is characterized by a wide variety of multiorgan injuries, among which hallmarks are proliferative glomerulonephritis and arthritis. In contrast, PD-1 deficiency in the BALB/C mouse strain results in dilated cardiomyopathy, which is mediated by antibodies to troponin I²⁵. PD-1 deficiency in other genetic backgrounds results in organ-specific autoimmunity depending on the type of autoimmunity to which each mouse strain is prone^{26–28}. The natural role of PD-1 in organ-specific peripheral tolerance was confirmed by the observation that distinct single nucleotide polymorphisms of the *PDCDI* are associated with distinct types of autoimmune diseases including SLE, rheumatoid arthritis, ankylosing spondylitis, multiple sclerosis, type I diabetes and Grave's disease^{29, 30}.

In contrast to the important beneficial role in maintaining peripheral tolerance and T cell homeostasis, PD-1 mediates potent inhibitory signals after ligation by PD-1 ligands expressed on malignant tumors and this effect has detrimental effects on anti-tumor immunity^{31–33}. Moreover, expression of PD-1 by “exhausted” virus-specific T cells that are characteristic of chronic viral infections prevents the proliferation and function of virus-specific T effector cells and clearance of the virus^{34, 35}. Although the role of PD-1 in peripheral tolerance, anti-viral and anti-tumor immunity is well established, little is known about how PD-1 ligation exerts its effects on specific signaling targets and how these altered signaling events impact on various aspects of T cell function.

Structure of PD-1

PD-1 consists of a single N-terminal IgV-like domain, an approximately 20 amino acid stalk separating the IgV domain from the plasma membrane, a transmembrane domain, and a cytoplasmic tail containing tyrosine-based signaling motifs. Due to the similarity of this structure with the structure of ICOS and CTLA-4, PD-1 was initially considered as a member of the CD28 superfamily. However, subsequently it became clear that PD-1 has distinct features that distant it from the classical members of the CD28 family (reviewed in 36). Specifically, CD28, CTLA-4 and ICOS have Src homology (SH2)-binding motifs (YxxM) located in the center of their cytoplasmic tails. CTLA4 and CD28 also have one and two SH3-binding motifs (PxxP), respectively, in their cytoplasmic tails. However, no such SH2-binding or SH3-binding motifs are present in the cytoplasmic tail of PD-1. Instead, the cytoplasmic tail of PD-1 contains an N-terminal sequence VDYGEL, forming an immunoreceptor tyrosine-based inhibition motif (ITIM), defined as V/I/LxYxxL, which recruits src homology-2 (SH2) domain containing phosphatases³⁷. The cytoplasmic tail of PD-1 also contains a C-terminal sequence TEYATI, forming an immunoreceptor tyrosine-based switch motif (ITSM), defined as TxYxxL (Figure 1). Such structure has been described in CD2 superfamily members³⁸ and in the CD33-related Siglec family, which mediated inhibitory functions in neuronal cells³⁹. Interestingly, the spacing between the ITIM and the ITSM motifs in the cytoplasmic tail of PD-1 and that of Siglec family members is conserved suggesting that spacing between these motifs will have an important functional role. An additional difference between PD-1 and the members of the CD28 family with possible functional implications, albeit currently unclear, is that CD28, ICOS and CTLA4 exist as dimers, whereas PD-1 exists as a monomer^{40, 41}.

Mechanisms of PD-1-mediated signal inhibition

The ITSM of PD-1 has a critical role in PD-1 mediated inhibitory function. Specifically, the ITSM tyrosine (Y248) of PD-1 associates with SHP-2 and is mandatory for initiation of PD-1-mediated inhibition of PI3K/Akt activation^{3, 42} (Figure 2). To study the role of the specific tyrosines in the cytoplasmic tail of PD-1 in primary human T cells, a chimeric CD28.PD-1 construct was generated, in which the extracellular domain of mouse CD28 was fused to the cytoplasmic tail of human PD-1 and was expressed in primary human T cells using a lentiviral vector-mediated transduction. This approach allowed dissecting the signals initiated via PD-1 containing an intact cytoplasmic tail or mutagenized forms of PD-1 cytoplasmic tail. Triggering of PD-1 mediated signals by using an anti-mouse CD28 mAb in

primary human T cells expressing each of these chimeric constructs showed inhibition of PI3K/Akt activation and impaired IL-2 production upon stimulation via CD3 and CD28. These inhibitory effects on PI3K/Akt activation and IL-2 production were preserved when the ITIM tyrosine (Y223) in PD-1 cytoplasmic tail was mutagenized to phenylalanine (Y223F) but were lost when the ITSM tyrosine (Y248) was mutagenized (Y248F). In that system, the presence of Y248F mutation abrogated the interaction of PD-1 with SHP-2^{3, 42}.

The role of tyrosine Y248 in mediating the inhibitory effects of PD-1 in T cell activation is further supported by other studies, which employed different approaches. Specifically, PD-1 engagement inhibited B-cell receptor (BCR)-mediated Ca²⁺ mobilization and phosphorylation of Igβ, Syk, PLC-γ2 and Erk1/2, and these effects were dependent on SHP-2 recruitment to the ITSM tyrosine of PD-1⁹. In addition, mass spectrometry studies to identify molecules interacting with phosphorylated PD-1 cytoplasmic domains showed that a phosphorylated peptide corresponding to ITSM of PD-1 could serve as a docking site of SHP-2 *in vitro*⁴³. In contrast to the previous work performed in live cells³, this *in vitro* approach found that interaction with SHP-2 could also be detected when a phosphorylated peptide corresponding to ITIM of PD-1 was used for *in vitro* pulldown of interacting partners⁴³. However, an interaction of the ITIM domain of PD-1 cytoplasmic tail with SHP-2 has not been documented *in vivo*.

Another puzzling question has been the role of SHP-2 versus SHP-1 in the inhibitory function of PD-1. Although the role of SHP-2 in mediating the inhibitory functions of PD-1 is well documented, it has been reported that SHP-1 can also be detected in the context of PD-1-mediated inhibition of T cell activation. Specifically, SHP-1 would be recruited to the ITSM of PD-1 cytoplasmic tail in the system of mouse/human CD28.PD-1 chimeric receptor³. Similarly to SHP-2, SHP-1 was also detected to interact with a phosphopeptide corresponding to the PD-1 ITSM using mass spectrometry⁴³. Using live cell imaging it was determined recently that SHP-2 and not SHP-1 is the phosphatase, which interacts with PD-1 upon TCR mediated activation⁴⁴. With this elegant system, the mechanism via which PD-1 inhibits T cell activation was studied in real time. This work revealed that PD-1 is translocated to dynamic TCR microclusters and accumulates at the signaling central supramolecular activation cluster (c-SMAC). SHP-2 is immediately recruited to PD-1 in the microclusters and associates with the ITSM of PD-1. Recruitment of SHP-2 to PD-1 correlated with dephosphorylation of TCR proximal signaling molecules within the PD-1-TCR microclusters. These studies provided evidence that localization of PD-1 within the TCR microclusters is required for the PD-1-SHP-2 association and PD-1-mediated inhibition of T cell activation. These findings suggest that PD-1, a molecule lacking enzymatic activity or direct interaction with enzymatically active molecules, becomes phosphorylated by protein tyrosine kinases that are activated upon TCR ligation and explain why PD-1 exerts its inhibitory function only upon simultaneous TCR ligation.

Although these compelling studies provided evidence that SHP-2 is colocalizes with PD-1 in the cSMAC and interacts with the ITSM of PD-1, these findings do not preclude a potential role of ITIM in the function of PD-1. Consistent with this hypothesis, using site directed mutagenesis and stable expression of mutagenized PD-1 constructs in Jurkat T cells, it was determined that although only mutation of Y248 abrogated interaction with SHP-2, both

Y248 and Y223 were actively involved in the inhibitory effects of PD-1 on IL-2 production. Specifically, in T cells expressing either PD-1.Y223F or PD-1.Y248F, ligation of PD-1 with PD-L1-Ig fusion protein during TCR/CD3-mediated activation diminished IL-2 production by 50% but ligation of PD-1 in T cells expressing the double mutant PD-1.Y223F/Y248F almost abrogated IL-2 production⁴⁵. These results indicate that although phosphorylation-dependent interactions of PD-1 with SHP-2 involve Y248 in the ITSM, yet unidentified interactions of Y223 in the ITIM are mandatory for PD-1-mediated inhibitory function on T cell activation.

Effects of PD-1 ligation on TCR downstream biochemical signaling

The first identified target of PD-1-mediated inhibitory function in T cells was the PI3K/Akt pathway. Interestingly, PD-1 ligation inhibits activation of the PI3K/Akt pathway in a manner distinct from that induced by CTLA-4. Specifically, it has been proposed that PD-1 blocks the CD28-mediated activation of phosphatidylinositol-3-kinase (PI3K) by recruiting SHP-2. In contrast, CTLA-4 directly inhibits Akt activation, but not PI3K activation, possibly by the association with protein phosphatase 2A (PP2A)⁴². However, CTLA-4 can also interact with SHP-2 and/or SHP-1⁴⁶. Furthermore, PP2A binds CTLA-4 but also CD28⁴⁷, which mediates a positive signal upon TCR-mediated activation. Considering the complex and non-specific interactions of these phosphatases with stimulatory and inhibitory co-receptors, other mechanisms than differential recruitment of phosphatases might regulate PD-1-mediated inhibition of PI3K/Akt activation.

Recently, it was determined that at least one mechanism via which PD-1 inhibits activation of the PI3K/Akt pathway involves PTEN phosphorylation and phosphatase activity, mediated by CK2⁴⁸. PTEN is a serine/threonine phosphoprotein and a substrate of CK2 *in vitro* and *in vivo*. CK2 mediates phosphorylation of PTEN C-terminus serine/threonine cluster S380/T382/T383, which promotes PTEN protein stability, while reducing PTEN lipid phosphatase activity against its substrate PIP3^{49, 50}. During TCR/CD3 and CD28-mediated stimulation, PTEN is phosphorylated by CK2 in the Ser380/Thr382/Thr383 cluster within the C-terminus regulatory domain⁴⁸. CK2-mediated phosphorylation on the C-terminus cluster stabilizes PTEN, resulting in increased protein abundance due to resistance to ubiquitin-dependent degradation, but suppressed PTEN phosphatase activity. PD-1 inhibits the stabilizing phosphorylation of the Ser380/Thr382/Thr383 cluster within the C-terminus domain of PTEN, thereby resulting in diminished abundance of PTEN protein but increased PTEN phosphatase activity (Figure 2). Remarkably, the CK2 phosphorylation sites in PTEN are conserved in species from mammals to *Xenopus laevis*, and clusters of putative CK2 phosphorylation sites are also present at the C-terminus of PTEN in *Drosophila melanogaster*, *Caenorhabditis elegans*, and *Saccharomyces cerevisiae*⁵¹. Thus, CK2-mediated phosphorylation of PTEN represents a dominant mechanism, which regulates PTEN phosphatase activity, and this mechanism is directly targeted by PD-1 to mediate its inhibitory effect on the PI3K/Akt pathway in T cells.

The second major signaling pathway, which is targeted by PD-1, is the Ras/MEK/Erk pathway^{43, 52}. The major mechanism responsible for activation of Ras and its downstream MEK/Erk MAP kinase pathway in T cells involves the Ca²⁺- and DAG (CalDAG)-mediated

activation of RasGRP1^{53–55}, which are activated downstream of PLC- γ 1⁵⁶ (Figure 2). Studies in primary human T cells revealed that MEK/Erk MAP pathway is a target of PD-1, an event secondary to inhibition of PLC- γ 1 and Ras activation. The effect of PD-1 on MEK/Erk MAP kinases was selective because PD-1 ligation did not inhibit the activation of Jnk and p38 MAP kinases⁵². Although the PI3K/Akt and Ras/MEK/Erk pathways have been the center of attention as targets of PD-1, other signaling events initiated by TCR ligation are also attenuated by PD-1 ligation including ZAP70 and PKC θ activation⁴³.

Functional implications of altered biochemical signaling induced by PD-1

PD-1 targets components of cell cycle machinery

A major downstream target of the synergistic effect of PI3K/Akt and Ras/MEK/Erk activation in T cells is the cell cycle machinery. Primary T lymphocytes naturally reside in the G0 phase and lack expression of cyclins, which are required to interact with cyclin-dependent kinases (Cdks) in order to form cyclin-Cdk holoenzyme complexes that drive cell cycle progression^{57–59}. Expression of D1-type cyclins occurs during entry into G1 phase, expression of cyclin E at the late G1 restriction point, and expression of cyclin A at the S phase. p27^{kip1}, a member of the Kip/Cip family of Cdk inhibitors is abundantly expressed in T cells and interacts with Cdk2. Initiation of cell cycle progression in T cells requires ubiquitin-dependent degradation of p27^{kip1} thereby allowing activation of Cdk2 and entry to the S phase of the cell cycle. This event is regulated by Skp1-Cullin-F-box (SCF) family ubiquitin ligase, SCF^{skp2}⁶⁰. TCR/CD3 and CD28 stimulation mediates transcriptional induction of Skp2, the substrate recognition component of the SCF^{skp2} ubiquitin ligase, and this induction of Skp2 expression requires simultaneous activation of both PI3K/Akt and Ras/MEK/Erk pathways⁶¹. In contrast, p38 MAP kinase has no effect on the expression of Skp2 and on the degradation of p27^{kip1} upon T cell activation. PD-1 during the T cell stimulation inhibited activation of the PI3K-Akt and Ras/MEK/ERK pathways, expression of Skp2 was abrogated resulting in elevated levels of p27^{kip1} and inhibition of Cdk2. Because of the impaired activity of Cdk2, T cells stimulated through PD-1 not only displayed decreased phosphorylation of Rb but also failed to phosphorylate the checkpoint inhibitor Smad3 on the Cdk2-specific site, leading to enhanced Smad3 transcriptional activity⁶², which resulted in the increased abundance of the G1 phase Cdk inhibitor, p15^{INK4B}, and the abrogation of the Cdk-activating phosphatase Cdc25A⁶³.

These findings identified how PD-1 inhibits cell cycle progression but also revealed an unexpected mechanism by which PD-1 might affect the fate of T cells by regulating Cdk2, which interacts with many signaling pathways and regulates multiple functional outcomes. In conjunction with cyclin E, Cdk2 phosphorylates the cell cycle inhibitor p27^{kip1} resulting in ubiquitin-targeted degradation, as mentioned above. Cdk2 promotes phosphorylation of Rb on specific sites thereby reversing its ability to sequester E2F⁶⁴. Cdk2-mediated phosphorylation of Rb also impacts the interactions of Rb with histone deacetylases and other chromatin remodeling proteins^{65, 66}. Cdk2-cyclin E also phosphorylates a number of substrates, which affect histone gene expression, centrosome duplication and replication origin licensing^{67, 68}. Cdk2 directly regulates expression of genes including NF κ B, Sp1, p300/CBP, and subunits of the RNA polymerase reviewed in⁶⁹. Cdk2 also phosphorylates

Smad3 and antagonizes its antiproliferative function induced by TGF- β whereas impaired phosphorylation on the cdk-specific sites renders Smad3 more effective in executing its antiproliferative function⁶². Thus, inhibition of Cdk2 activation by PD-1 may lead to differential phosphorylation of such Cdk2 substrates, resulting in a distinct program of gene expression, differentiation and function of T cells. Thus, due to its effects on Cdk2, PD-1 may regulate T cell properties and function by reprogramming transcriptional and epigenetic events independently of its effects as an inhibitor of cell cycle progression.

Synergistic signaling between PD-1 and TGF- β and implications on iTreg generation

PD-1 reduces the threshold of TGF- β -mediated signals thereby synergizing with TGF- β to promote the conversion of naïve T cells into inducible Treg (iTreg) cells. Specifically, PD-1 can mediate the formation of iTregs in the presence of minimal amounts of TGF- β or even in the absence of exogenous TGF- β ²³. Subsequent studies indicated that this effect of PD-1 was not due to inducing increased production of TGF- β or enhanced expression of TGF- β receptors. Instead the synergistic effect of PD-1 with TGF- β in the generation of iTreg was due to inhibition of Cdk2-mediated phosphorylation of Smad3, which resulted in an enhanced Smad3 transactivation in a TGF- β -independent manner. Thus, PD-1 synergizes with TGF- β -mediated signals at a level distal to the TGF- β receptor and regulates TGF- β -specific transcriptional events by directly regulating the function of Smad3. This synergizing effect of PD-1 with TGF- β signaling on naïve T cells promotes the differentiation of Treg cells thereby mediating suppression of T effector cells via a cell extrinsic mechanism. In addition, this effect can facilitate direct suppression of T effector cells via an intrinsic mechanism.

Generation of Treg cells requires the α L β 2 (LFA-1) integrin^{70, 71}. Inside-out signaling regulates attainment of the active, high affinity conformation of β 2 integrin, which depends on activation of the small GTPase Rap1⁷². Integrin activation is mandatory for the function of Treg cells⁷³. Importantly, PD-1 does not inhibit TCR-mediated activation of Rap1, in contrast to targeting Ras⁵², indicating that PD-1 not only promotes the generation of Treg cells but supports pathways required for Treg cells to execute their immunosuppressive function.

PD-1 alters the metabolic reprogramming of activated T cells

Upon activation, naïve T cells undergo a metabolic reprogramming to glycolysis, which is required so support their growth, proliferation and effector functions^{74, 75}. Signals from the CD28 costimulatory pathway and the γ -chain signaling cytokines promote this metabolic program^{76, 77}. Divergence in the metabolic reprogramming of T cells is critical to imprint distinct T cell fates. This has been shown with the switch to glycolysis that accompanies effector T cell differentiation⁷⁸ and the switch to fatty acid β -oxidation (FAO) that accompanies the conversion of T effector to T memory cells⁷⁹. Furthermore, enforcing FAO by pharmacologic means promotes the generation of Treg cells⁸⁰. We investigated the metabolism of T cells receiving PD-1 signals and we discovered that they were unable to engage in glycolysis, glutaminolysis or metabolism of branched chain amino acids but displayed increased rate of fatty acid oxidation (FAO)⁸¹. In contrast to inhibiting expression of receptors and enzymes involved in uptake and metabolism of glucose, glutamine and

branched amino acids, PD-1 increased the expression of carnitine palmitoyl transferase (CPT1A), the rate-limiting enzyme of FAO (Figure 2). We determined that extracellular acidification rate (ECAR), an indicator of glycolysis, and oxygen consumption rate (OCR), an indicator of oxidative phosphorylation (OXPHOS), were increased upon T cell activation. Activated T cells receiving PD-1 signals had lower ECAR and OCR but higher OCR/ECAR ratio compared to T cells stimulated without PD-1 ligation. These findings indicated that in contrast to proliferating T cells, which preferentially use glycolysis for energy production, T cells receiving PD-1 signals are rather metabolically quiescent and preferentially use OXPHOS than glycolysis as indicated by the higher OCR/ECAR ratio. These findings indicate that PD-1 ligation alters the metabolic reprogramming induced upon T cell activation by inhibiting glycolysis and promoting FAO.

Concluding remarks

PD-1, initially considered a molecule regulating cell death, is now identified as a dominant inhibitory receptor, which alters the function of T cells upon antigen-specific stimulation. These altered responses result from inhibition of multiple signaling events initiated upon TCR ligation. PD-1-mediated altered signaling results in inhibition of T effector cell responses by cell intrinsic mechanisms. PD-1 impacts on the generation and expansion of T effector cells by targeting PI3K/Akt and Ras/MEK/Erk pathways both of which are required for the transcription of effector cytokines and for activation of cell cycle progression through transcriptional induction of Skp2. The effects of PD-1 on biochemical signaling pathways also promote the generation of iTreg cells via multiple mechanisms. First, by suppressing Akt activation, PD-1 promotes Foxp3 expression. Second, by inhibiting Cdk2, PD-1 enhances TGF- β -mediated transactivation of Smad3, thereby promoting Foxp3 transcription. Third, by suppressing glycolysis and promoting FAO, PD-1 specifically activates a metabolic program that promotes generation of Treg cells, while suppressing the generation of Th1 and Th17 cells. PD-1 does not mediate global inhibition of signaling events initiated upon T cell activation because certain biochemical targets such as activation of JNK and p38 MAP kinases and Rap1 GTPase remain unaffected by PD-1 ligation. The imbalanced activation of signaling pathways induced by PD-1 results in altered metabolic programs, differentiation profiles and functional properties of T cells with a net outcome of T cell quiescence and immune suppression. Better understanding of the biochemical signaling effects of PD-1 will shed light on the molecular mechanisms that are responsible for PD-1-mediated hyporesponsiveness in patients with chronic infections and cancer. Understanding of such signaling effects will also provide insight into the mechanisms responsible for the break of tolerance in patients with autoimmune diseases. Ultimately, such knowledge will guide the design of combinatorial therapies to modulate PD-1 and its downstream targets with the goal to improve immunity in chronic infections and cancer or to induce tolerance in the context of autoimmune diseases and allogeneic transplantation.

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MQIPQAPWPVVWAVLQLGWRPGWFLDSPDRPWNPPTFSPALLVVTEGDNATFTCSFSNTS
 1 60
 ESFVLNWMSPSNQTDKLAAPEDRSQPGQDCRFRVTQLPNGRDFHMSVVRARRNDSGT
 120
 YLCGAISLAPKAQIKESLRAELRVTERRAEVPTAHPSPSPRPAGQFQTLVVGVVGGLLGS
 180
 LVLLVWVLAVICSR AARGTIGARRTGQPLKEDPSAVPVFSVDYGEIDFQWREKTPEPPVP
 223 240
 CVPEQTEYATIVFP SGMGTSSPARRGSADGPRSAQPLRPEDGHCSWPL
 248

Figure 1. Amino acid sequence of human PD-1

Numbers below each line refer to the amino acid position. The IgV-like domain is underlined. The signals sequence (grey), transmembrane segment (blue), the ITIM and the ITSM motifs (green), and the tyrosines within these motifs (yellow) are highlighted.

Figure 2. Schematic presentation of the biochemical signaling altered by PD-1 and its functional implications

Binding of SHP2 on ITSM and a yet unidentified partner on ITM of PD-1 inhibits TCR-mediated activation of the PI3K/Akt pathway and the PLC-γ1/Ras/MEK/Erk1/2 pathway. As a consequence, T cells are unable to progress in the S phase of the cell cycle, to produce cytokines and genes responsible for the activation and differentiation programs initiated by TCR ligation. PD-1 has a major effect on the metabolic reprogramming of activated T cells by suppressing glycolysis and promoting FAO. This altered metabolic reprogramming impacts on the differentiation program of T cells by preventing generation of T effector cells and promoting generation of Treg cells.