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A rheostat for immune responses: the unique properties of PD-1 and their advantages for clinical application

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PD-1, a negative coreceptor expressed on antigen-stimulated T cells and B cells, seems to serve as a 'rheostat' of the immune response. The molecular mechanisms of the functions of PD-1, in conjunction with the mild, chronic and strain-specific autoimmune phenotypes of PD-1-deficient mice, in contrast to the devastating fatal autoimmune disease of mice deficient in the immunomodulatory receptor CTLA-4, suggest that immunoregulation by PD-1 is rather antigen specific and is mainly cell intrinsic. Such unique properties make PD-1 a powerful target for immunological therapy, with highly effective clinical applications for cancer treatment.

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Antigen receptors are known to have a broad specificity with a wide range of affinity. This intrinsic characteristic of antigen receptors inevitably makes thymic negative selection incomplete for the avoidance of self-reactive immune responses in the periphery. It is therefore essential that the antigen-recognition signaling system be equipped with a 'rheostat' that regulates the threshold of antigen responses for balanced physiology of the immune system. Deficiency in PD-1 (CD279), a negative coreceptor of unknown function initially isolated by cDNA subtraction[1], results in the development of different autoimmune phenotypes on various genetic backgrounds of mice[2–7]. PD-1 is thus a critical negative coreceptor that regulates the threshold of antigen responses of T cells and B cells in the periphery. The activation of PD-1 after it interacts with its ligands[8,9] dephosphorylates key proteins immediately downstream of the antigen receptor[10–14], which endows PD-1 with immunoregulatory functions.

By regulating the function of $CD8^+$ T cells, PD-1 modulates immunity to infection. Deficiency in PD-1 renders mice resistant to viral infection by reducing the antigen-recognition threshold and increasing the cytotoxic lymphocyte activity of $CD8^+$ T cells. The proposal of such a regulatory effect of PD-1 on the threshold of T cell immune responses is supported by the observation that deficiency in PD-1 induces the suppression of tumor growth and tumor metastasis in mice[15,16]. Furthermore, PD-1 is essential for the generation and selection of high-quality, high-affinity antibodies by regulating the properties and abundance of antigen-stimulated $CD4^+$ T cells[17,18]. Such regulation is critical not only for antibody-mediated memory but also for control of the gut microbiota by the adaptive immune system. The latter aspect opens a new perspective on the involvement of PD-1 in the fine-tuned regulation of symbiotic relationships between the immune system and the gut microbiota and in the regulation of other physiological systems of the body through interaction with the entire range of microbial products.

PD-1 has unique properties relative to those of CTLA-4 (CD152), the other major negative coreceptor expressed on T cells. CTLA-4 has both cell-intrinsic activities (on effector cells) and cell-extrinsic activities (on $Foxp3^+$ T cells), which make the autoimmune phenotypes of deficiency in CTLA-4 very severe and antigen nonspecific[19,20]. In contrast, the effects of engaging PD-1 are mainly cell intrinsic. The cell-intrinsic function of PD-1, as well as the regulation of PD-1 expression, are probably responsible for the relatively milder and more chronic pathological phenotypes that result from PD-1 blockade by either antibody or genetic manipulation. The subtle effects of PD-1 blockade are now being exploited in translational medicine for boosting immune responses in several pathologies.

In this Review, we discuss the unique properties of PD-1 as a 'rheostat' of immunological regulation. We focus on the characteristics that make PD-1 distinct from other negative regulators (coinhibitory receptors, transcription factors or cytokines) and discuss the immunological mechanisms that endow PD-1 with unique modulatory functions critical for immunological therapy against tumors. We present an overview of the research into PD-1 over the course of the past two decades and highlight how the discovery of PD-1 led to an appreciation of the delicate regulation of antigen stimulation for immunophysiology and its exciting application to tumor treatment.

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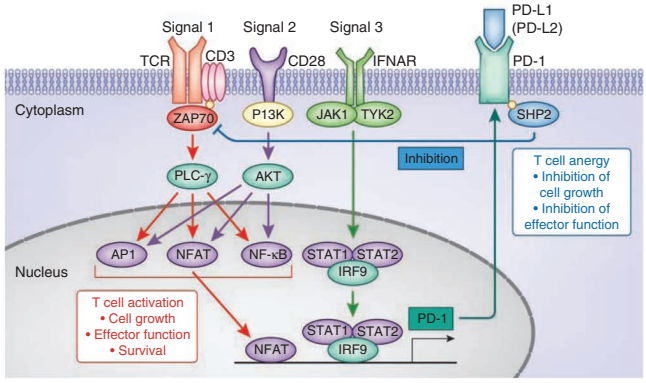

Figure 1 PD-1 induces T cell tolerance. PD-1 inhibits the TCR signaling pathway through SHP-2. T cells are activated by signal 1 (antigen stimulation), signal 2 (costimulation) and signal 3 (inflammatory cytokines). In naive T cells, TCR-mediated calcium influx initiates *Pdcd1* transcription by activating NFATc1. In chronically activated ('exhausted') T cells, interferon-α (IFN-α) causes prolonged *Pdcd1* transcription through the binding of the transcription factor IRF9 to the *Pdcd1* promoter. When its physiological ligand (PD-L1 or PD-L2) binds, PD-1 suppresses the activation and function of T cells through the recruitment of SHP-2, which dephosphorylates and inactivates Zap70, a major integrator of TCR-mediated signaling. CD3, coreceptor; PI(3)K, Jak1 and Tyk2, kinases; PLC-γ, phospholipase C-γ; Akt, kinase; NF-κB, transcription factor.

At present, PD-1 offers one of the best examples of scientific translation from bench to bedside and a powerful demonstration to all—scientists, pharmaceutical companies and funding agencies—of the extreme importance of basic research for progress in medicine.

PD-1 regulates the threshold of immune responses

The activation of lymphocytes depends mainly on the recognition of antigen by antigen-specific receptors, whereas additional inputs through coreceptors fine tune that activation signal to regulate its strength, duration and properties. The fate of lymphocytes after antigen encounter is determined by the integration of stimulatory and inhibitory signals from coreceptors, each with unique characteristics. CD28 and CTLA-4, which provide positive and negative costimulation, respectively, upon interaction with either of two shared ligands, B7.1 (CD80) and B7.2 (CD86), are the prototypes of such coreceptors. PD-1, together with several other proteins such as ICOS (CD278) and BTLA (CD272), belongs to the CD28 coreceptor family. Like CD28 and CTLA-4, PD-1 has two ligands, PD-L1 (B7-H1 or CD274)[8] and PD-L2 (B7-DC or CD273)[9]. PD-1 lacks the membrane-proximal cysteine residue required for homodimerization, which is characteristic of other members of the CD28 family. Thus, PD-1 exist as a monomer on the cell surface[21–23]. Because of its interaction with the adaptor complex AP2, CTLA-4 is subject to continuous clathrin-dependent endocytosis and is therefore almost undetectable on the cell surface[24]. In contrast, PD-1 lacks an AP2-binding motif, which may allow its sustained expression on the surface of activated T cells.

Engagement of PD-1 by ligand during antigen recognition induces crosslinkage of the antigen-receptor complex with PD-1. That results in phosphorylation of the tyrosine residue in the immunoreceptor tyrosine-based switch motif (TxYxxL/I, where 'x' indicates any amino acid and 'L/I' indicates 'leucine or isoleucine') of PD-1 and recruits the tyrosine phosphatase SHP-2, which dephosphorylates and inactivates proximal effector molecules such as Syk in B cells and Zap70 in T cells[10–14]. The immediate outcome of stimulation via PD-1 is inhibition of cell growth and cytokine secretion (**Fig. 1**).

PD-1 is expressed on $CD4^-CD8^-$ double-negative αβ and γδ T cells in thymus and on activated T cells, B cells, natural killer cells, natural killer T cells and myeloid cells in the periphery[25–29]. Activation-induced expression of PD-1 suggests that PD-1-dependent inhibition functions in later phases of the immune response (for example, sustained activation, secondary responses, effector phases, etc.)[25]. When naive DO11.10 T cells (which have transgenic expression of an ovalbumin-specific T cell antigen receptor (TCR)) from PD-1-deficient and PD-1-sufficient mice are stimulated with $PD\text{-}L1^+$ antigen-presenting cells *in vitro*, PD-1 deficiency results in a greater number of cells with more than three divisions but not those with one to two divisions, which suggests that PD-1 expression is induced during the first to second round of division, after which T cells became sensitive to PD-L1 on antigen-presenting cells[30]. PD-1 begins to function after T cells recognize their cognate antigen and start the activation process. The amount and source of antigen determine the strength and kinetics of T cell activation and thus the amount and kinetics of PD-1 expression. In addition, the expression of PD-1 ligands also varies depending on the cell type and their activation status. Therefore, PD-1-dependent inhibition is very sensitive to the context, and thus the antigen, which may explain why deficiency in PD-1 apparently augments antigen-specific immune responses, although neither PD-1 nor its ligands are antigen specific.

Anatomical variation in the expression of PD-1 ligands critically determines where and when PD-1 functions. There is high expression of PD-L1 on nonlymphoid cells, including parenchymal cells, tumor cells and virus-infected cells, which allows PD-1 to directly inhibit effector functions against target cells (**Fig. 2**). In an *in vitro* experiment, PD-L1-expressing tumor cells have shown resistance to cytotoxicity, which suggests that the engagement of PD-1 on $CD8^+$ T cells by PD-L1 expressed on specific target cells results in the inhibition of T cell–mediated cytotoxic activity[15]. In autoimmunity, PD-L1 is induced on tissue-parenchymal cells in the affected organs[4,31]. In experiments involving bone marrow chimeras and adoptive transfer, PD-L1 expression on parenchymal cells rather than on hematopoietic cells has been shown to protect against autoimmune diabetes[28]. In contrast to the broad expression of PD-L1 in many tissues, the expression of PD-L2 is restricted to professional antigen-presenting cells (dendritic cells, B cells, etc.)[27]. PD-L2 might be involved in various aspects of immunoregulation, such as T cell–B cell 'collaboration' for the production of appropriate antibodies (discussed below).

Although most experimental data support the idea that PD-1 inhibits antigen stimulation in a cell-intrinsic manner after interacting with either of its two ligands, there are several observations that could be explained by alternative mechanisms, including reverse signaling through PD-1 ligands[32]. However, the physiological contributions of such alternative pathways are unclear at present, in part because no distinct molecular mechanisms have been identified.

PD-1 regulates peripheral tolerance

Deficiency in PD-1 results in the loss of peripheral tolerance and the subsequent development of autoimmunity in mice[2,3]. Aged PD-1-deficient ($Pdcd1^{-/-}$) mice develop lupus-like glomerulonephritis and arthritis on a C57BL/6 background, whereas they develop dilated cardiomyopathy through the generation of antibodies to troponin I on the BALB/c background[3,33]. Those observations were the first clear experimental demonstration of the autoimmune basis of dilated cardiomyopathy and provided the rationale for immuno-adsorption therapy for this deadly disease[34]. The tissue-damaging autoantibodies produced in BALB/c $Pdcd1^{-/-}$ mice require class switching and/or

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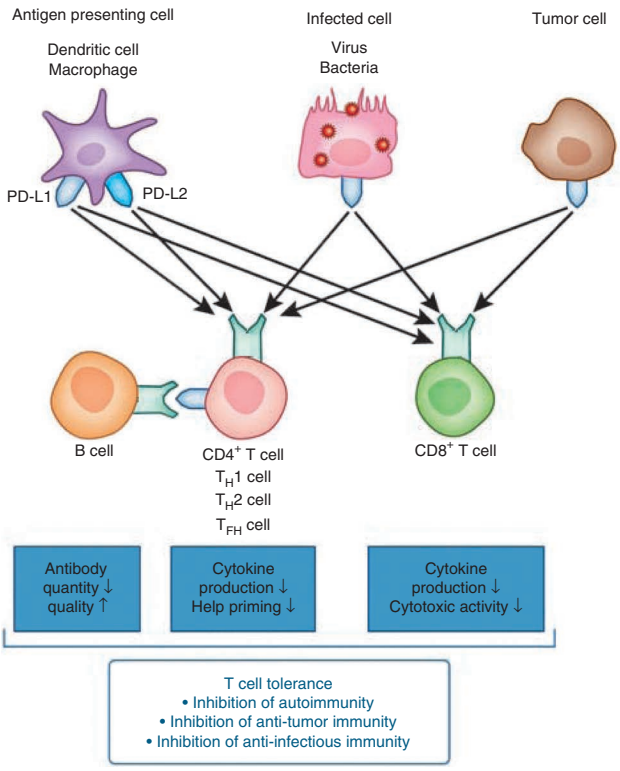

Figure 2 Biological importance of PD-1 signaling. PD-1 is induced on activated CD4+ T cells and CD8+ T cells, as well as on B cells. Inflammatory stimulation induces PD-L1 expression on many types of nonhematopoietic and hematopoietic cells and PD-L2 expression on antigen-presenting cells. The engagement of PD-1 with its ligands inhibits the proliferation and effector function of T cells and antibody production of B cells, which results in the prevention of autoimmunity and attenuation of antitumor and anti-infectious immunity.

somatic hypermutation, because the development of dilated cardiomyopathy and gastritis in such mice is dependent on the cytidine deaminase AID, a master regulator of class switching and somatic hypermutation[30]. As discussed below, the self-reactive antibodies in PD-1-deficient mice are probably generated in germinal centers (GCs) induced systemically by dysregulated gut microbiota and are aided by GC T cells with proinflammatory properties (i.e., cells that produce more interferon-γ but less interleukin 21 (IL-21) than do PD-1-sufficient GC T cells)[18].

In addition, backcrossing of *Pdcd1*$^{-/-}$ mice on various other backgrounds has revealed that deficiency in PD-1 induces autoimmunity in diverse target organs depending on the genetic background of the mice[4–7]. The finding that variations in the disease phenotype depend on the genetic background suggests that the absence of PD-1 may exaggerate strain-specific susceptibilities to autoimmune disorders. That indicates that regulation by PD-1 is apparently (that is, not absolutely) antigen specific. The target specificity of the PD-1-dependent regulation of autoimmunity also holds true for the association between single-nucleotide polymorphisms in *PDCD1* and various kinds of human autoimmune diseases, including systemic lupus erythematosus, type I diabetes, multiple sclerosis, rheumatoid arthritis, Grave's disease and ankylosing spondylitis[35–37]. Some of those diseases are associated with the IL-17-producing T_H17 subset of effector helper T cells. In experimental autoimmune encephalitis, a model for human multiple sclerosis, PD-1 substantially downregulates expression of IL-6, a proinflammatory cytokine produced by cells of the innate immune system that is required for T_H17 differentiation[38]. Possibly PD-1 regulates both innate responses and lymphocyte responses to maintain self-tolerance.

PD-1 acts together with another inhibitory coreceptor, LAG-3, in the regulation of autoimmunity[30]. Mice deficient in both LAG-3 and PD-1 on the BALB/c background die of autoimmune myocarditis by 5 weeks of age. *In vitro* experiments have revealed that PD-1 and LAG-3 synergistically inhibit the antigen-induced activation of T lymphocytes. Although LAG-3 is proposed to suppress the activation of CD4+ T cells by competing with the coreceptor CD4 for binding major histocompatibility complex class II, the precise molecular mechanism for this and its biological function remain largely unknown[39,40].

Distinct physiological functions of PD-1 and CTLA-4

As already mentioned above, the autoimmune phenotypes of *Pdcd1*$^{-/-}$ mice are generally much milder and more confined to specific organs and have a rather later onset than those of *Ctla4*$^{-/-}$ mice. In the latter mice, T cells that are nonspecifically activated invade various organs, which results in premature death with graft-versus-host–like disease regardless of the genetic background[20]. That extremely deleterious phenotype is reminiscent of mice deficient in the transcription factor Foxp3 and transforming growth factor-β1 (TGF-β1)[41–43]. In fact, >90% of CTLA-4-expressing cells also express Foxp3 (ref. 20). Interestingly, the severely deleterious phenotypes of mice deficient in CTLA-4, TGF-β or Foxp3 appear to be caused mainly by cell-extrinsic mechanisms, given the following observations: cotransfer of CTLA-4-deficient bone marrow cells along with wild-type bone marrow cells 'rescues' the autoimmune phenotype caused by CTLA-4-deficient bone marrow cells alone[44]; adoptive transfer of bone marrow cells from wild-type mice 'rescues' the lethal phenotype of Foxp3-deficient *scurfy* mice[45]; and TGF-β1 inhibits inflammatory cells and promotes the development and function of Foxp3+ T cells[46]. In contrast, transfer of PD-1-deficient splenocytes or bone marrow cells along with their respective wild-type counterparts into immunodeficient mice does not 'rescue' the autoimmune phenotypes caused by PD-1-deficient cells alone, which suggests that the phenotype of PD-1-deficient mice is attributable mainly to cell-intrinsic mechanisms[5,30] (**Fig. 3**).

A large number of reports have suggested that CTLA-4 serves critical roles in regulating immune responses mainly by modulating the function of Foxp3+ T cells[20]. However, several groups have reported that CTLA-4-deficient Foxp3+ T cells retain their suppressive functions, which suggests that various mechanisms may operate under different assay conditions. Similarly, conflicting data have been reported on whether Foxp3+ T cells from TGF-β1-deficient mice are able to suppress the autoimmune phenotypes of Foxp3-deficient mice[47]. It will be critical to clarify whether CTLA-4, Foxp3 and TGF-β1 function in parallel, in series or both for the negative regulation of immune responses.

Although Foxp3+ T cells express PD-1, the contribution of PD-1 to their suppressive function seems to be small, if any, at least in an *in vitro* suppression assay[6]. In fact, the Foxp3+ T cell compartment is larger in PD-1-deficient mice than in PD-1-sufficient mice (S.F., unpublished data). Since PD-1-deficient T cells are prone to be activated and Foxp3 expression is induced in activated T cells[48], it is reasonable that PD-1-deficient mice have a greater frequency of Foxp3+ T cells.

The inhibitory mechanisms of PD-1 and CTLA-4 are distinct, as CTLA-4 completely blocks costimulation by CD28 through its stronger affinity for B7 molecules[49,50], whereas the inhibitory effect of PD-1 on signaling through the TCR and costimulation by CD28 is indirect and is thus less complete and slower. The inhibitory function

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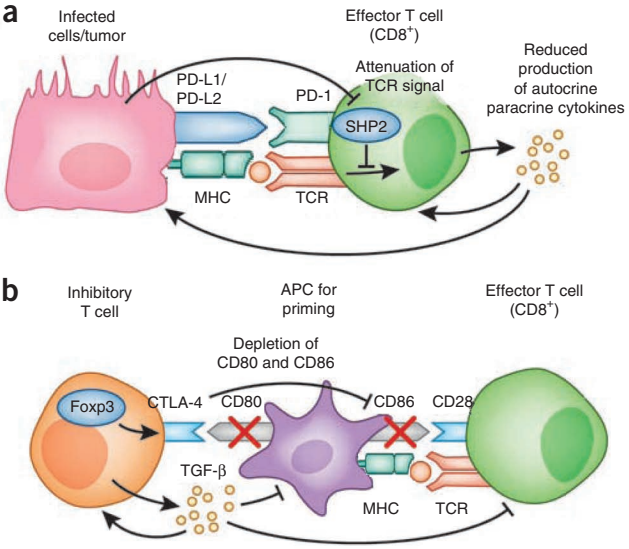

Figure 3 Distinct mechanisms of PD-1 and CTLA4 in immunosuppression. (**a**) PD-1 controls the effector phase of the immunity in a mainly cell-intrinsic manner by inducing unresponsiveness through attenuating antigen-specific signals. Autocrine and/or paracrine regulation by PD-1 is also possible by inhibiting cytokine expression. Only $CD8^+$ T cells are included here. Although it is not shown here, $CD4^+$ T cells interact with antigen-presenting B cells and regulate the quality and quantity of antibodies. MHC, major histocompatibility complex. (**b**) CTLA-4 controls, in particular, the function of activated $CD4^+$ T cells that express Foxp3. CTLA-4 dominantly captures CD80 and CD86 on antigen-presenting cell (APC) and down-modulates the costimulatory activity of CD28 on effector T cells. TGF-β1, an important cytokine produced by $Foxp3^+$ T cells, supports the growth and differentiation of $Foxp3^+$ T cells and suppresses diverse immune responses.

of PD-1 depends mostly on its recruitment of SHP-2, whereas CTLA-4 signaling involves a wider variety of molecules, including SHP-2, the phosphatase PP2A and AP2 (ref. 12). The AP2-dependent endocytosis of CTLA-4 together with the B7 ligands has been suggested to be central in the regulatory function of $Foxp3^+$ cells[51]. Although it remains to be seen whether that represents the main mechanism for the cell-extrinsic effects of CTLA-4, it is likely that CTLA-4 has both cell-intrinsic and cell-extrinsic effects and functions as an 'on-off', rather than a 'rheostat', negative regulator. Thus, the inhibitory functions of PD-1 appear to be distinct from those of other negative regulators, with PD-1 deficiency affecting antigen-specific autoimmune responses, whereas deficiency in other negative regulators results in more systemic, non–antigen-specific phenotypes.

Regulation of antibody responses by PD-1

Humoral immune responses are generated by T cell–independent and T cell–dependent pathways. Interestingly, both types of immune responses are controlled by PD-1. There is high expression of PD-1 by innate-type B cells, such as peritoneal cavity B-1 cells, after their activation by antigens[52,53], and PD-1 seems to suppress the population expansion of B-1 cells induced by the B cell antigen receptor and their differentiation into long-lived plasma cells that express immunoglobulin G (IgG)[54]. Thus, the engagement of PD-1 on B-1 cells by its ligands (probably expressed by macrophages located in the red pulp of the spleen, where B-1 cell–derived plasmablasts 'preferentially' locate, and by the plasma cells themselves) contributes to the down-modulation of innate-like responses and facilitates the 'takeover' by the adaptive responses through longer-lived plasma cells and memory cells generated in the GC[55].

GCs are specialized microenvironments in which antigen-activated B cells interacting with T cells upregulate AID expression and undergo the two genetic alterations required for effective and long-lasting immune responses: class-switch recombination and somatic hypermutation[56]. PD-1 seems to regulate those two GC-dependent immunological functions. There is high expression of PD-1 on the two subsets of $CD4^+$ T cells present in the GCs: follicular helper T cells (T_{FH} cells), defined as $CXCR5^+PD\text{-}1^{hi}Foxp3^-$ cells[57], and follicular regulatory T cells (T_{FR} cells), defined as $CXCR5^+PD\text{-}1^{hi}Foxp3^+$ cells[58–60]. Some GC B cells also express PD-1 (ref. 17), while PD-L1 and PD-L2 are expressed in the GCs, with the B cells in the light zone of the GCs and memory B cells having high PD-L2 expression[17,18]. Thus, PD-1 on GC T cells is engaged mainly by PD-L2 expressed on GC B cells, and that interaction probably modulates the GC reaction *in situ*. Indeed, the B cell–intrinsic expression of PD-L2 is required for the optimal generation of antibody-secreting cells[17]. However, the generation of antibody-secreting cells by mice deficient in both PD-L1 and PD-L2 or of $Pdcd1^{-/-}$mice is more severely impaired than that of mice deficient in PD-L2 alone, at least after systemic immunization with nitrophenol conjugated to chicken γ-globulin. Thus, constitutive PD-L1 expression on B cells also probably contributes to PD-1 signaling and affects systemic GC responses. A lack of interactions between PD-1 and its ligands results in inappropriate immune responses, manifested by reduced formation of long-lived IgG^+ plasma cells in the bone morrow[17] and production of IgA antibodies with poor bacteria-binding properties in the gut[18].

Mechanistically, PD-1-deficiency in mice leads to the population expansion of T_{FH} cells with reduced ability to produce IL-21 (refs. 17,18). IL-21 is important for the formation and function of GCs, and its absence affects the proliferation of B cells and their differentiation into memory B cells and plasma cells[61–63]. Deficiency in PD-1 results in enhanced production of other cytokines by $CD4^+$ T cells located outside GCs (i.e., pre-GC T cells), which may suppress the optimal production of IL-21 by T_{FH} cells. IL-2, one of the cytokines regulated by PD-1 (ref. 64), is known to inhibit the generation and function of T_{FH} cells (through regulation of the expression of the transcriptional repressor Blimp-1 mediated by the transcription factor STAT5)[65,66].

The finding that PD-1 might regulate T_{FR} cells (i.e., deficiency in PD-1 may enhance the abundance and suppressive functions of T_{FR} cells) further adds to the complex role of PD-1 in regulating the GC reaction[67]. Regardless of the regulatory mechanisms (directly, through T_{FH} cells, or indirectly, through T_{FR} cells), lack of interaction between PD-1 and its ligands mitigates the GC response and leads to impaired immunological memory, defective selection of plasma cells and an imbalance in bacterial communities in the gut. Microbial dysbiosis in $Pdcd1^{-/-}$ mice impairs the gut barrier function and leads to generalized activation of the immune system, which drives the population expansion of self-reactive B cells and T cells and the production of autoantibodies[18,30]. Such findings emphasize the critical role of PD-1 in the regulation of not only T cell responses but also B cell responses. Under homeostatic conditions, the 'rheostat' function of PD-1 is critical for maintaining the balance of bacterial communities in gut, while during infection it is essential for the generation of immunological memory (**Fig. 2**). By influencing the composition of the gut microbiota, PD-1 probably contributes to the fine-tuning of other major physiological processes in the body, such as the function of the endocrine system, cardiovascular system or nervous system.

Regulation of viral infection by PD-1

PD-1 has unique regulatory roles in the control of viral infections. PD-1 clearly attenuates the magnitude of primary responses during

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acute infection. In a model of adenovirus-induced hepatitis, deficiency in PD-1 has been shown to augment the proliferation and accumulation of effector T cells in the liver and cause rapid clearance of the adenovirus[68] (**Fig. 2**). However, despite that early clearance of virus, the *Pdcd1*$^{-/-}$ mice develop severe hepatitis, in contrast to wild-type mice, which develop prolonged hepatitis with slow viral clearance. Such observations indicate that PD-1 may be important for avoiding excessive tissue damage during the acute phase of infection. Consistent with that idea, infection of PD-L1-deficient mice with lymphocytic choriomeningitis virus (LCMV) clone 13, known to cause viral persistence, results in death of the infected mice due to severe damage to the liver[69].

Although transient PD-1 expression may inhibit excessive immune response during acute infection, high and persistent expression of PD-1 results in chronic immune responses. Interestingly, extremely high expression of PD-1 (about two to three logs higher than that of transiently activated T cells) has been observed on CD8$^+$ T cells during chronic infection with LCMV clone 13 (ref. 69). In that study, virus-specific PD-1^{hi}CD8$^+$ T cells were shown to have fallen into a state of anergy or unresponsiveness and therefore were called 'exhausted' T cells (to emphasize their lost ability to produce tumor-necrosis factor, interferon-γ and IL-2)[69]. That 'exhausted' PD-1^{hi} population was not functionally incompetent but contained memory cells capable of population reexpansion and viral clearance after secondary infection with a nonpersisting (acute) clone of LCMV, Armstrong strain. Interestingly, the reexpanded 'exhausted' population retained high PD-1 expression and low expression of cytokines, which suggests that the 'exhaustion' is established as a consequence of PD-1-mediated adaptation to pathogens without self damage, resulting in chronic infection[70]. Notably, transient blockade of the PD-1–PD-L1 pathway by monoclonal antibody (mAb) to PD-L1 restored the function of 'exhausted T cells' and enhanced T cell responses for clearing the viruses. Such reversal of the exhausted state by blockade of PD-L1 can be accomplished even in 'helpless' mice depleted of CD4$^+$ T cells, which suggests that the recovery of cytotoxic properties is intrinsic to CD8$^+$ T cells[69]. Similar high expression of PD-1 is observed in CD4$^+$ T cells from tumor-bearing and aged mice[71]. Although those senescent PD-1^{hi} CD4$^+$ T cells proliferate poorly in response to stimulation via the TCR, they still produce osteopontin (a proinflammatory cytokine) and have high expression of C/EBP-α (a regulator normally expressed on cells of the myeloid lineage) and diminished expression of the cell-cycle regulators c-Myc and cyclin D1.

The high expression of PD-1 during the chronic phases of immunological reactions may be achieved by substantial and irreversible demethylation of CpG dinucleotides in the promoter region of *Pdcd1* located 500–1,500 base pairs upstream of the initiation codon[72]. In addition, that region contains two transcription factor–binding sites (for NFAT, at position –1160 relative to the transcription start site[73,74], and ISRE, at position –1040)[73], which are activated by TCR- and interferon-dependent pathways, respectively. It is likely that continuous stimulation through antigen receptors (the TCR and B cell antigen receptor), acting together with inflammatory cytokines (i.e., interferons) causes the demethylation (opening) of the locus, which results in high expression of PD-1.

As blockade of PD-1 after the establishment of chronic infection 'refuels' the immune response, PD-1 seems a likely target for therapy directed against chronic infection. However, the outcome of such PD-1 blockade varies depending on the experimental system and the timing of the blockade. Further analysis should provide a better rationale for the safe and effective application of a PD-1 antagonist for the treatment of chronic infection as well as the improvement of immune responses after vaccination, or for the use of a PD-1 agonist for the treatment of fulminant diseases.

Regulation of tumor growth and metastasis by PD-1

The PD-1–PD-L1 pathway has a pivotal role in dampening immunosurveillance for tumors. The first evidence of this was provided by the observation that overexpression of PD-L1 on a mouse plasmacytoma cell line inhibits the cytolytic activity of CD8$^+$ T cells through engagement of PD-1, which enhances their growth and invasiveness[15]. In addition, that report clearly demonstrated that PD-L1$^+$ myeloma cells do not produce tumors in *Pdcd1*$^{-/-}$ mice and that blockade of the interaction between PD-L1 and PD-1 activates CD8$^+$ T cells that can attack tumors (**Fig. 2**). On the other hand, expression of PD-L1 on tumors is reported to provide resistance to T cells by promoting the apoptosis of T cells through non-PD-1 receptors[75].

So far, blockade of the PD-1–PD-L1 interaction by various systems, including antibody blockade of PD-1 and PD-L1, genetic manipulation of PD-1 and vaccination with DNA encoding the extracellular region of PD-1, has been shown to accelerate tumor eradication[15,76–79]. Accumulated data from clinical samples have shown that high expression of PD-1 ligands on tumors correlates with poor prognosis, which suggests that tumors could escape antitumor immunity through engagement of ligand by PD-1 on T cells[80,81].

On the basis of those results, a fully humanized mAb to PD-1 (nivolumab; also known as ONO4538, MDX-1106 or BMS 936558) has been developed through the immunization, with human PD-1, of genetically modified mice carrying loci encoding human immunoglobulins[82]. The IgG4 isotype of nivolumab minimizes complement activation or antibody-dependent cell-mediated cytotoxicity and thus avoids unnecessary cellular toxicity and inflammation. The phase I clinical trial study of nivolumab was initiated in 2006 (ref. 82). The cumulative response rates were 18% (14 of 76 patients) for non–small-cell lung cancer, 28% (26 of 94 patients) for melanoma and 27% (9 of 33 patients) for renal cell carcinoma[83]. Drug-related adverse events of grades 3–4 occurred in 14 patients. A clinical trial using a mAb to PD-L1 (BMS-936559 or MDX-1105) showed similar anticancer activity[84]. In those studies, blockade of PD-1 pathway (by either mAb to PD-1 or mAb to PD-L1) showed the highest rate of antitumor activity of the many immunotherapy approaches tested in the clinic for the treatment of cancer during the past 30 years[85]. Accumulating data suggest that the therapeutic use of mAb to PD-1 alone or in combination with other drugs will provide successful therapy for many types of advanced cancer. Nivolumab has been designated an 'orphan drug' for malignant melanoma (http://www.accessdata.fda.gov/scripts/opdlisting/oopd/OOPD_Results_2.cfm?Index_Number=387612).

Concluding remarks

A key point in elucidating the function of PD-1 as a 'rheostat' of the immune response has been the characterization of the autoimmune phenotype of PD-1-deficient mice, with manifestations that are unique and strikingly different among different genetic backgrounds[2–7]. The background- and organ-specific autoimmune manifestations, together with the relatively mild symptoms and late disease onset, indicate that PD-1 might regulate the immune response in an antigen-specific fashion. That assumption is supported by additional molecular studies revealing the role of PD-1 in inhibiting signal transduction after specific engagement of antigen receptors. Elucidation of the molecular mechanisms of the functions of PD-1, together with the identification of its ligands[8,9], has allowed analysis of the involvement of PD-1 in various aspects of the immune response.

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Another critical observation is that blockade of PD-1–PD-L1 interactions by antibody or genetic manipulation can potentiate $CD8^+$ T cells' attacking tumors and viruses[15,16,68]. That finding eventually led to clinical studies investigating the association between ligand expression on tumors and the prognosis of patients with tumors[81]. The most decisively beneficial result has been the finding that blocking PD-1 through the use antibodies is so far the most promising immunotherapy for cancers. That development, in turn, indicates that the immune system effectively recognizes tumor cells and suggests that the rekindling of immunosurveillance, which is probably made anergic by a large excess of tumor antigens and chronic stimulation[86], may be an effective strategy for controlling malignancies. The success of blocking PD-1 in cancer treatment appears to depend on the unique features of PD-1 discussed briefly in this Review, which clearly distinguish PD-1 from other molecules with immunosuppressor functions.

Nevertheless, it is important to recall that at least 70% of patients with cancer have not responded well to treatment with antibody to PD-1 (ref. 83). The exact reason for that unresponsiveness is a target for further investigation. Genetic polymorphisms and cancer cell mutations should be thoroughly investigated to identify clinical markers that could distinguish patients who respond from those who do not respond. It will also be important to assess various combination therapies for those who do not respond. Those combinations can include stimulation with cancer-targeting peptides and combined therapy with agents that block other negative regulators, with the expectation of either additive or synergistic effects on antitumor activities. Such complementary treatments include mAb to PD-1 plus cytokines (i.e., interferon-α)[73], antigen stimulation[87] and blockade of other immunoinhibitory pathways, such as those of Tim-3 (ref. 88), LAG-3 (ref. 89) and CTLA-4 (ref. 90). A combinational therapy of mAb to PD-1 and mAb to CTLA-4 has resulted in an objective response rate of more than 50% for patients with advanced melanoma[89]. Overall, the serendipitous journey of PD-1 from bench to bedside once again teaches that basic scientific research is critical for drug discovery and translational medicine.

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The authors declare no competing financial interests.

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