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INVITED REVIEW ARTICLE

Basics of PD-1 in self-tolerance, infection, and cancer immunity

Shunsuke Chikuma[1]

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Abstract Successful cancer treatment requires understanding host immune response against tumor cells. PD-1 belongs to the CD28 superfamily of receptors that work as "checkpoints" of immune activation. PD-1 maintains immune self-tolerance to prevent autoimmunity and controls T-cell reaction during infection to prevent excessive tissue damage. Tumor cells that arise from normal tissue acquire mutations that can be targeted by lymphocytes. Accumulating lines of evidence suggest that tumor cells evade host immune attack by expressing physiological PD-1 ligands and stimulating PD-1 on the lymphocytes. Based on this idea, researchers have successfully demonstrated that systemic administration of monoclonal antibodies that inhibit the binding of PD-1 to the ligands reactivated T cells and augmented the anti-cancer immune response. In this review, I summarize the basics of T-cell biology and its regulation by PD-1 and discuss the current understanding and questions about this multifaceted molecule.

Keywords PD-1 · CTLA-4 · Immune checkpoint blockade · Immune tolerance · Costimulation · T lymphocytes

PD-1 and PD-ligand as costimulatory molecules

CD28–CTLA-4 system

The acquired immune system, mediated by T and B lymphocytes, provides lifelong protection against pathogens. The T-lymphocyte (T-cell) reaction is triggered by the recognition of an antigen on the major histocompatibility complex (MHC) by a T-cell antigen receptor (TCR). In the steady state, all the nucleated cells express self-antigens on MHC and can potentially activate self-reactive T cells. Most self-reactive T cells are eliminated in the thymus through a mechanism called "negative selection"; however, many of them escape selection and are present in the periphery. Thus, there must be systems that prevent activation of autoreactive T cells in the periphery.

It is well characterized that the first signal, provided through the TCR during recognition, does not alone cause activation of the T cells. Full T-cell activation requires a second set of signals, called "costimulation," which is mainly provided by activated antigen-presenting cells (APCs) (Fig. 1). The best characterized costimulatory system is the CD28 receptor on T cells, triggered by its ligands, CD80 and CD86 (old names: B7-1 and B7-2, respectively) on activated professional APCs (reviewed by Lenschow et al. [1]). CD80 and CD86 are upregulated on activated APCs by microbial "danger signals" so that the APCs presenting microbial antigens can efficiently stimulate T cells for activation (Fig. 1b). However, resting APCs do not express CD80 and CD86. T cells that are stimulated in the absence of the CD28 signal shift to an unresponsive state called "clonal anergy" and become refractory to further stimulation by the same antigen (Fig. 1a). CTLA-4, another receptor structurally similar to CD28, is induced on activated T cells and binds to CD80 and CD86 with greater avidity than does CD28 (Fig. 1c) (reviewed by Bour-Jordan et al. [2]). When CTLA-4 was discovered, its function gained much attention because it could have been either a positive or negative feedback regulator for T-cell activation. Monoclonal antibodies (mAbs) that block binding of CTLA-4 to CD80/CD86 are shown to augment T-cell

✉ Shunsuke Chikuma
schikuma@keio.jp

[1] Department of Microbiology and Immunology, Keio University School of Medicine, 35 Shinanomachi, East Lecture Hall 4F, Shinjuku-ku, Tokyo 160-8582, Japan

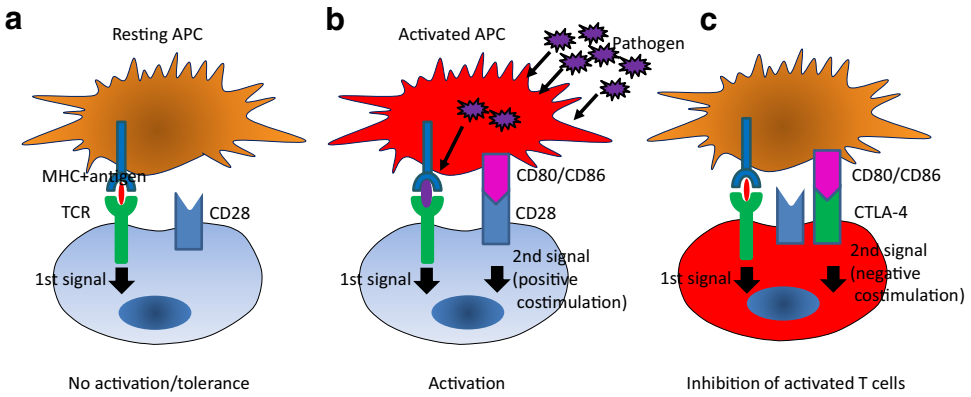

Fig. 1 Basis for T-cell tolerance by costimulatory molecules. **a** In the steady state, antigen-presenting cells (APCs) preferentially express autoantigens. T cells stimulated without CD28 ligation fall into anergy, which avoids unwanted activation. **b** In infection, pathogens activate APCs through innate receptors, leading to expression of costimulatory ligands (CD80/CD86). CD80 and CD86 can engage CD28, allowing full activation of pathogen-specific T cells. **c** Activated T cells express CTLA-4. CTLA-4 binds to CD80/CD86 with high affinity and provide negative regulation of T cells

activation when added into the coculture of T cells, APCs, and antigen in a soluble form [3, 4]. From this experiment, CTLA-4 was suggested to be a negative regulator for T-cell activation. The CTLA-4 knockout (KO) mice generated separately by three groups supported this notion [5–7]. All the germline CTLA-4 KO mice showed massive lymphocyte proliferation in the lymph nodes and spleen, followed by autoimmune attack against virtually all tissues by lymphocytes, and premature death [5, 6]. The phenotype clearly suggested CTLA-4 provides negative feedback on T-cell activation. Thus, T-cell activation against self is fundamentally regulated by two "checkpoints." (1) T cells in the absence of CD28 signal (in the absence of CD80 and CD86 on APCs) become unresponsive (Fig. 1a). (2) Upon activation, CTLA-4 is induced, ligated by CD80/CD86, and prevents further activation of self-reactive T cells (Fig. 1c). After this discovery, several pairs of ligands and receptors were reported and were shown to have unique functions in the immune system (Fig. 2) [8]. PD-1 belongs to the CD28 family and provides such an "immune checkpoint" at the priming and effector phases of immunity.

Cloning and characterization of PD-1 and PD-1 ligands

PD-1 (CD279) was originally reported by Ishida et al. [9] as a molecule induced on cells undergoing apoptosis and hence named "Programmed cell-death 1." Structurally, PD-1 possesses an immunoglobulin V-like domain on its ectodomain and a short cytoplasmic tail. The structure resembles CD28 and other molecules in the same family (Fig. 2). In contrast to CD28 or CTLA-4, PD-1 lacks a motif for homodimerization and is expressed as a monomeric form on the plasma membrane. Additionally, PD-1 possesses a unique immunoreceptor-tyrosine based

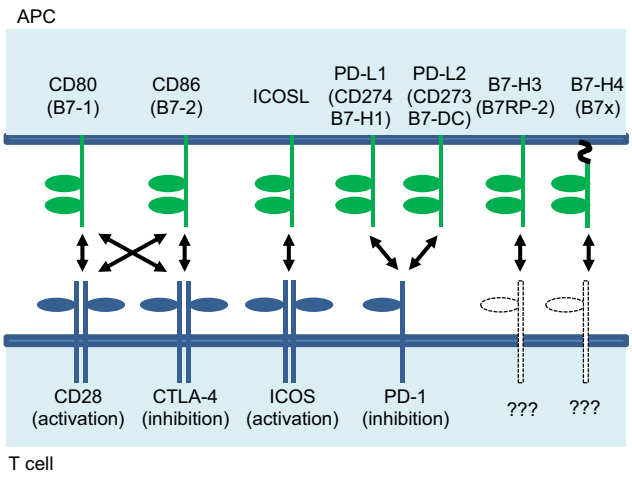

Fig. 2 Receptors and ligands of the CD28-B7 family

inhibitory motif (ITIM), which was later identified as an essential motif for the function of PD-1. PD-1 expression is found mostly on leukocytes and is induced upon their activation [10]. PD-1 is absent or very low on naïve T cells and is induced upon TCR stimulation. In vivo, a cognate peptide antigen for T cells induces PD-1 in a very short time (~6 h) after challenge [11]. Physiological PD-1 expression is developmentally regulated [12]. Immature thymocytes, gamma-delta (γδ) T cells, natural killer T cells, and innate lymphoid cells constitutively and strongly express PD-1. B cells also express PD-1, and there are some reports supporting a cell-intrinsic role of PD-1 in regulation of B cells [13].

Two ligands for PD-1 were identified based on homology search or cDNA subtraction [14–17]. The molecules named PD-L1 (alternative name; B7-H1, CD274) and

PD-L2 (B7-DC, CD273) are both type 1 transmembrane proteins and possess two Ig-like domains, structurally similar to CD80, CD86, ICOS ligand, and so on (Fig. 2) [8], and selectively bind to PD-1. In vitro studies demonstrated that engagement of PD-1 by both PD-L1 and PD-L2 inhibited T-cell proliferation and secretion of cytokines [interleukin (IL)-2, IL-4, interferon (IFN)-γ, and IL-10), suggesting these receptors similarly transmit inhibitory signals through PD-1 [14, 16]. However, the two ligands differ in their expression and in vivo function. PD-L1 is expressed on various organs, such as lung, heart, thymus, spleen, kidney, and liver [14, 15]. At the cellular level, many epithelial cells and leukocytes express PD-L1, which is further augmented by IFN-γ signaling [18]. The upregulation of PD-L1 by IFN-γ seems to be mediated by transcriptional factor STAT1 [19, 20], which possibly explains the high expression of PD-L1 in inflamed tissues and cancer. On the other hand, PD-L2 expression is largely restricted on APCs such as dendritic cells and macrophages [18] and is triggered by the NF-κb signal [21]. PD-L1 knockout (KO) mice phenocopy PD-1 KO in terms of enhanced lymphocyte reaction and susceptibility to autoimmune diseases [22, 23], but PD-L2 knockout mice do not [24]. The phenotypic difference is probably the result of PD-L1 expression on the MHC class I^+ target tissue, whereas PD-L2 can work only at the time of T-cell priming by professional APCs [24].

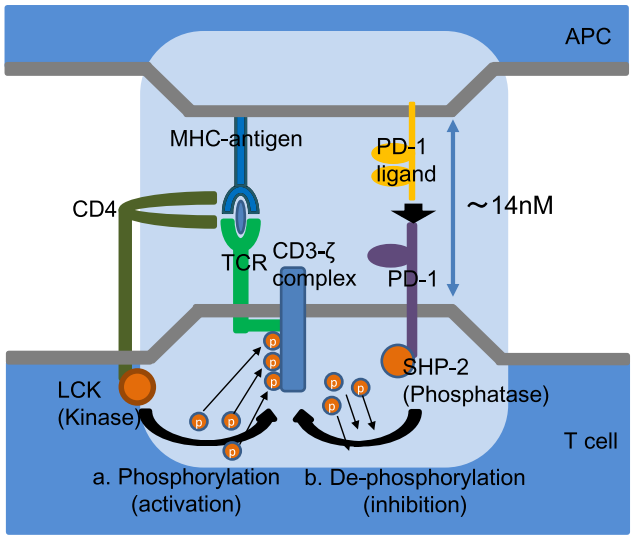

Fig. 3 A molecular model for Programmed Death (PD)-1-mediated inhibition of T cells. **a** Recognition of MHC–antigen complex by T-cell receptor (TCR) and CD4 leads to LCK kinase-mediated phosphorylation of the CD3-TCRζ complex, initiating downstream signals. **b** PD-1 ligation by PD-1 ligands brings PD-1 to the proximity of TCR, and the associated SHP-2 phosphatase dephosphorylates the CD3-TCRζ complex, leading to the attenuation of signal. The inhibition occurs within a 3D structure created between TCR and APC

Immune checkpoint inhibition by PD-1: a self-tolerance point of view

PD-1 in self-tolerance

In 1998, a mouse carrying a germline null mutation of PD-1 was generated and was reported to develop a late-onset lupus-like autoimmune syndrome [25]. Differing in initial C57BL/6 background, PD-1 KO in BALB/c background were shown to develop lethal dilated cardiomyopathy [26], which was caused by autoantibody against troponin I, a myocardium-specific regulator of the actin-myosin system [27]. The data suggested that PD-1 regulates self-tolerance against many organs, depending on the genetic background of individuals (i.e., MHC haplotype). The phenotype of PD-1 KO mice is more obvious in mice carrying autoimmune susceptibility. In the NOD mice that develop spontaneous type I diabetes, null mutation of PD-1 [28] or ligands [24] accelerated the activation of islet-reactive T cells and resulted in much higher penetrance, earlier onset, and more severe diabetes progression than the PD-1-sufficient counterpart. The Murphy Roths Large (MRL/MpJ) mice are known to be prone to autoimmunity. MRL mice lacking either PD-1 [29] or PD-L1 [22] develop severe myocarditis and pneumonia, and more than 70 % of the mice die within the first 10 weeks of age. Interestingly, PD-1-sufficient MRL mice do not show myocarditis, suggesting the PD-1 null genotype provokes unrecognized host autoimmune predisposition.

The molecular basis of PD-1-mediated T-cell inhibition

How does PD-1 regulate T cells at the molecular level? To respond to numerous antigens, T cells and B cells carry antigen receptors with broad specificity. The intracellular region of the receptor complex converts the antigenic recognition into a digital signal (Fig. 3). In the case of T cells, the signal is initiated by the phosphorylation of CD3 molecules within the antigen–receptor complex by LCK, a tyrosine kinase associated with the coreceptors CD4 or CD8 (Fig. 3a). The phosphorylated tyrosine recruits nonreceptor tyrosine kinase zeta-associated protein 70 (ZAP70). Then, ZAP70 phosphorylates downstream adapter molecules, which transmit biochemical signals to the nucleus.

It is generally accepted that ITIM motif recruits several nonreceptor-type tyrosine phosphatases. PD-1 was found to recruit SH2-containing protein tyrosine phosphatase-2 (SHP-2) upon ligation by PD-1 ligands [16, 30–32]. The recruited phosphatase in turn dephosphorylates the cytoplasmic tail of CD3 molecules (Fig. 3b). Thus, PD-1 exerts its inhibitory effect by mediating the opposing reaction to

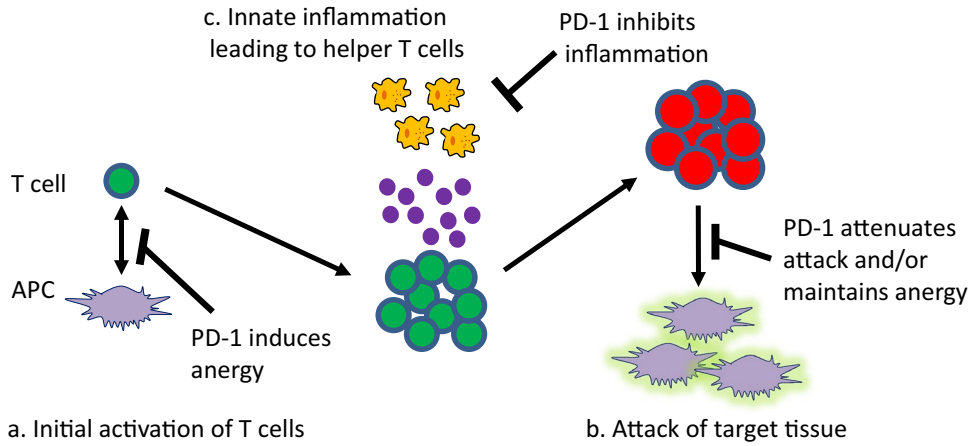

Fig. 4 Putative "immune checkpoints" by PD-1

the activation signal. The inhibition of TCR by PD-1 was demonstrated to occur within the T-cell–APC contact site termed the immunological synapse. Yokosuka et al., by using a high-resolution microscope, demonstrated that PD-1 enters into a "microcluster" that is formed within the immunological synapse [32]. The TCR and MHC peptide bear relatively small ectodomains; hence, they create a short intermembrane distance (~14 nm) at the time of antigen-induced synapse formation (Fig. 3). The small ectodomain of PD-1 and its ligands "fit" this distance and help them to enter the proximity of the TCR–peptide MHC complex, allowing efficient inhibition of the TCR signal during antigen recognition. At the transcriptional level, PD-1 ligation simply inhibited many genes that are triggered by TCR stimulation, rather than stimulating the de novo production of inhibitory molecules [31]. In short, PD-1 attenuates the TCR signal by antagonizing the biochemical reaction at the time of antigen recognition.

PD-1 as "immune-checkpoint" molecule

T-cell responses include initial activation of naïve antigen-specific T cells, clonal expansion, differentiation, and the effector phase. PD-1-mediated attenuation of TCR signaling seems to act as a "checkpoint" in these processes.

It was believed that a TCR signal without CD28-mediated costimulation caused incomplete T-cell activation, resulting in passive clonal anergy. However, this model was revised after several groups demonstrated that active inhibition by the PD-1–PD-L1 pathway contributes to this process (Fig. 4a). Upon antigen encounter without overt inflammation, naïve T cells rapidly express PD-1 and lapse into an unresponsive state. Naïve $CD8^+$ T cells with known specificity, when stimulated in vivo by cognate antigens, cannot respond when restimulated in vitro with the same antigen, which is a typical anergy [11, 33]. Such T cells, in the absence of PD-1 signal in vivo, did not fall into anergy and normally responded to the in vitro recall stimulation, which was demonstrated to be mediated by limitation of IL-2 expression by PD-1-mediated T-cell inhibition [11]. Next, PD-1 regulates autoimmune attack of effector T cells directly at the target site (Fig. 4b). As already mentioned, PD-1 is involved in attenuation of spontaneous autoimmune diabetes in NOD mice. Keir et al. [24] demonstrated that PD-L1, but not PD-L2, expressed on pancreatic beta cells was enough to delay the massive progression of insulitis in NOD mice. Fife et al. [34] showed $CD4^+$ T cells specific for pancreatic β-cell antigens could be experimentally tolerized in vivo by pretreating them with artificial APCs expressing the cognate antigen. Anti-PD-1 or anti-PD-L1, but not anti-PD-L2, reversed this tolerance weeks after the tolerogenic treatment, and the mice immediately developed massive pancreatitis, resulting in diabetes [34]. Using two-photon microscopy, which allows in vivo imaging of autoreactive T cells, it was demonstrated that after PD-1 blockade, T cells are rapidly stabilized onto APCs, providing tight communication with APCs [35]. These data suggested PD-1 prevented autoimmunity by setting at least two checkpoints, namely, at the induction and maintenance phases of the anergic state (Fig. 4a, b). In addition, PD-1 may modulate autoimmunity by inhibiting innate immune response (Fig. 4c). For autoimmune attack, T cells differentiate into effector cells, such as helper or killer cells. Inflammatory cytokines from innate immune cells (macrophages and dendritic cells) aid T-cell differentiation. Rui et al. [36] demonstrated that macrophages from PD-1 KO mice produced robust IL-6 upon recognition of heat-killed mycobacterial adjuvant, which augmented development of IL-17-producing helper T cells in the experimental autoimmune encephalitis model. Although the mechanism for negative regulation of the myeloid cells is currently unknown, the data suggested PD-1 involvement in the regulation of both T cells and the innate immune cells (Fig. 4c).

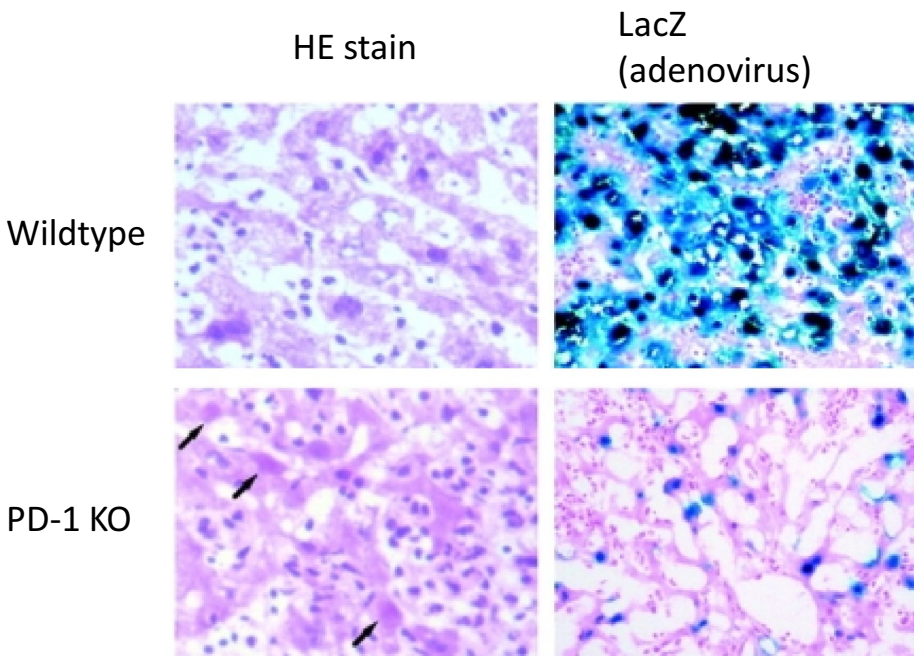

Fig. 5 PD-1 in virus infection. Livers of PD-1 KO mice 7 days after adeno-LacZ virus (adopted from Iwai et al. J Exp Med 2003 [37]). Compared to wild-type mice, PD-1 KO shows better clearance of virus (see *right panels*). Instead, PD-1 KO shows necrotic (*lower left panel*, *arrows*) and degenerated hepatocytes (*lower right panel*). *HE* hematoxylin and eosin stain

PD-1, T-cell exhaustion, and its role in infectious diseases

PD-1 is also important in the immune reaction during infection. As an early example, Iwai et al. [37] showed that experimental adenovirus, which causes acute infection in murine liver, was more rapidly eliminated in PD-1 KO mice than PD-1-sufficient mice (Fig. 5). Later, it was demonstrated that T cells show strong PD-1 expression during lymphocytic choriomeningitis virus (LCMV) [38], malaria [39], etc., pathogens that cause chronic infection. On the other hand, strong PD-L1 expression was observed in the parenchymal cells of the infected tissues. In this situation, the interaction between PD-1 on T cells and the ligand on infected cells was associated with the strongly anergic phenotype of T cells, called "exhaustion." The exhausted T cells show strong expression of inhibitory receptors (PD-1, TIM3, LAG3, etc.) and poor effector functions. The T-cell response in both acute and chronic infection is augmented via administration of blocking antibodies for PD-1–PD-L1 interaction, suggesting PD-1–PD-1 ligand interaction generally attenuates T-cell-mediated immune attack against pathogens.

One obvious question is why are PD-1 and the PD–ligand strongly induced and prevent beneficial virus clearance for the host? In the adenovirus model, despite the quick clearance of virus, the liver of PD-1 KO mice histologically showed severe degeneration (Fig. 5). It seems as though PD-1 protects the host by preventing a strong T-cell attack against infected liver cells. This idea was supported by an animal model of chronic infection. LCMV

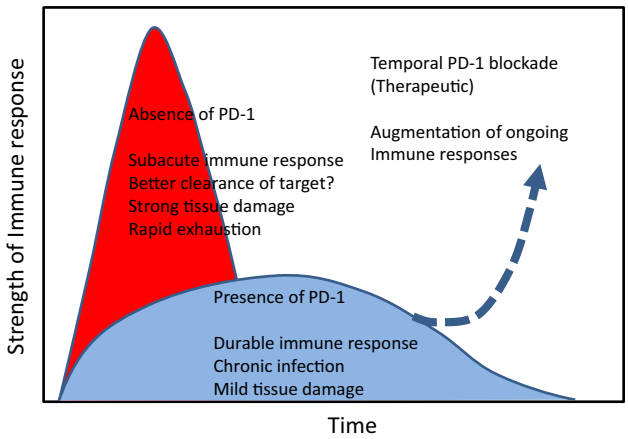

Fig. 6 A model for PD-1-mediated regulation of immune response

clone 13, in contrast to its parent strain, is known to cause persistent infection in mice. When PD-L1 knockout mice were infected by this virus, all the mice died of severe immune inflammation associated with T cells [38]. The same LCMV caused delayed clearance in the wild-type mice, which lasted until the eventual establishment of antiviral humoral immunity. Thus, PD-1 slows the course of immune response during infection, rather than choosing rapid tissue destruction (Fig. 6). PD-1 might have contributed to the establishment of the mutual existence of host and pathogens.

In this sense, it was interesting that several groups independently reported that PD-1 KO mice were very susceptible to mycobacteria, a persistent intracellular pathogen

[40–42]. Upon infection by mycobacteria, PD-1 KO mice developed a systemic cytokine storm and died. It should be pointed out that PD-1 KO mice could not control the mycobacterial burden at all. Recently, Odorizzi et al. [43] showed that T cells in PD-1 KO mice, at the site of infection, produced terminally differentiated effector T cells rapidly and lapsed into the exhausted phenotype (Fig. 6). These results challenge the idea that T cells become exhausted in a PD-1-dependent manner and suggest PD-1 is important for efficient control of infection. It is known that strong immune reaction causes production of terminally differentiated T cells that produce more cytokines but become functionally exhausted. PD-1 expression is induced at the site of T-cell recognition of the antigen, and flexibly attenuates the response [35, 44]. Thus, PD-1 is physiologically important for fine tuning the lymphocyte reaction to produce a beneficial pathogen clearance. Therefore, a temporal blockade (but not complete absence) of PD-1 during immune response may boost the ongoing immune response (Fig. 6 dotted arrow).

PD-1 blockade in cancer treatment

The basic concept

Tumor cells arise from normal tissue as a consequence of transformation. It is known that genomic mutation in the cancer creates mutated proteins that are presented on the MHC and are recognized by T cells as "neo-antigens." Therefore, theoretically the immune system can view transformed cells as "non-self." In spite of the immune surveillance, clinically apparent cancer develops, indicating evasion of immune attack. In mice, tumors developed in immunocompetent mice are more resistant to immune attack when transplanted to other mice than those developed in immunodeficient mice. This phenomenon, called "cancer immune editing," occurs as an eventual establishment of tolerance during the course of anti-cancer immune response in the body.

The idea of the immune checkpoint blockade in cancer was elegantly proposed by Jim Allison's group in 1996 [45], which demonstrated the systemic administration of mice with anti-CTLA-4 boosted anti-tumor response, resulting in the rejection of tumor. The research provided the first evidence that the blockade of the binding of the negative costimulatory molecule to its physiological ligand promotes tumor immunity, and thus established the concept of the immune checkpoint blockade in cancer treatment.

PD-1 blockade therapy for cancer treatment

Early studies showed that PD-L1 is frequently expressed on human cancer cells. Many groups separately reported that the level of PD-L1 expression significantly correlated with the poor prognosis of patients with various kinds of tumor (e.g., renal [46], gastric [47], urothelial [48], ovarian [49], and melanoma [50]). In mice, ectopic expression of PD-L1 on a tumor cell line inhibited the cytotoxic activity of killer T cells through PD-1 ligation [51]. These observations led to the idea that PD-L1 on the cancer cells triggers PD-1 on the attacking T cells, prevents their activation, and contributes to the induction of cancer immune tolerance. Researchers successfully confirmed this hypothesis by therapeutic administration of mice bearing tumor cell lines with anti-PD-1 and/or anti-PD-L1 antibodies [51, 52]. The first phase I clinical trial for a fully humanized mAb to PD-1 was reported in 2010 [53]. In 2012, data from a large-scale clinical study (~300 patients) were reported, demonstrating that monotherapy by either anti-PD-L1 [54] or PD-1 [55] resulted in up to 25 % overall response rate (ORR) in the initial clinical studies. The detailed clinical research of anti-PD-1 is reviewed by Dr. Hamanishi later in this issue.

Conclusion and perspectives

The therapeutic potential of anti-PD-1 therapy is unlimited at this point. Combinational therapies of anti-PD-1 and currently available cancer treatments offer new hope for many patients with various kinds of cancer. These therapies include chemotherapy, cancer vaccination, in vitro expansion of tumor-specific T-cell clones, irradiation, cytokine therapy, and combination with other immunotherapies. We recently showed that IFN-α triggers unnecessary PD-1 expression, which reduced the anti-tumor activity of IFN-α in a mouse study [56]. Compensating this "downside" of IFN-α by a combinational PD-1 blockade overcame this defect and resulted in rejection of tumors in most mice treated with IFN-α. In clinics, a combinational therapy of anti-CTLA-4 and PD-1 resulted in more than 60 % ORR in patients with melanoma, compared to the efficacy (~30 %) shown by therapy alone [57]. It is now being accepted that PD-1 and CTLA-4 may "check" different parts of the immune checkpoints; namely, CTLA-4 fundamentally regulates initial T-cell activation, whereas PD-1 mainly regulates the immune attack at the site of immune effector response [58]. Anti-PD-1, together with the blockade of other immune inhibitory pathways, causes a powerful boost for the immune reaction against a tumor. On the other hand, the immediate need is to avoid unnecessary immune response in the patients. Currently, the known adverse effects of anti-PD-1 include type I diabetes [59, 60], skin rash [55], myasthenia gravis [61], intestinal inflammation [55], and interstitial pneumonitis [55], some of which resulted in death for only a small number of patients but

too many to be ignored. Genetic background and immune status are the most likely candidates to determine the immunological outcome for a treatment. In this sense, it is interesting to observe that PD-1 knockout mice respond to some microbial factors very sensitively, sometimes resulting in enhancement of autoimmunity [36]. Consideration of the immunological backgrounds of patients (i.e., infection, allergy) should be beneficial to avoid side effects.

As already discussed, PD-1 might have evolved to optimize immune response to inhibit tissue damage during infection; however, it also causes unwanted immune inhibition in tumors. Vaccine development has defeated many lethal infectious diseases, bringing longevity, but longevity in turn gives rise to many cancers during the lifespan. Manipulation of the immune system by inhibiting the naturally developed inhibitory pathways, such as PD-1 and CTLA-4, will result in a good therapy for the enhancement of anti-cancer immunity.

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Compliance with ethical standard

Conflict of interest The author declares that I have no conflict of interest.

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