PD-1 regulates passive anaphylaxis: a possible role of the mast cell intracellular inhibitory signal

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To the Editor:

Antigen binding to a receptor initiates a cascade of intracellular signaling pathways and efficiently mounts an adaptive immune response in a given tissue microenvironment. Immunoreceptor engagement activates cytoplasmic protein tyrosine kinases such as SRC proto-oncogene (SRC), LYN proto-oncogene (LYN), and spleen associated tyrosine kinase. These kinases phosphorylate the immunoreceptor tyrosine-based activation motif (ITAM) and activate downstream immune effector functions through their interaction with the cytoplasmic tail. However, excessive activation can result in immune-mediated tissue damage. The cytoplasmic tail of programmed cell death-1 (PD-1) harbors an immunoreceptor tyrosine-based inhibitory motif (ITIM) and an immunoreceptor tyrosine-based switch motif (ITSM).[1] Like ITAM, these highly conserved motifs are phosphorylated upon immunoreceptor engagement[2] and interact with protein tyrosine phosphatase non-receptor type 6 or protein tyrosine phosphatase non-receptor type 11 (SHP-2). It is known that PD-1 interacts with SHP-2 and regulates antigen receptor signaling pathways through the cytoplasmic tail.[3, 4]

The mast cell (MC) is not only a key component of innate immunity but also serves as a critical effector in the adaptive immune response. Analogous to the T/B cell receptor signaling pathways, the high-affinity immunoglobin E receptor (FcεRI) signaling pathway involves ITAM phosphorylation, which in turn results in degranulation and the release of mediators/enzymes. Conversely, the ITIM/ITSM phosphorylation event negatively regulates the MC effector function.[5]Although it was recently shown that the ligation of PD-1 on MCs induces peripheral tolerance,[6] there has been a paucity of direct evidence showing the inhibitory role of PD-1 on MC effector function *in vivo* . To this end, we employed the IgE-mediated passive anaphylaxis model. We compared the consequences of total PD-1 absence with PD-1 receptor blockade[7] and delineated the requirement of the cytoplasmic tail for the regulation of FcεRI signaling.

In contrast to the findings for MC-specific deletion of SHP-2,[8] cutaneous, or peritoneal MC populations in PD-1 knockout (PD-1KO) mice were not altered significantly (Fig. S1a, b). This discrepancy suggests that PD-1 is dispensable for the development of the tissue MC lineage on the KIT proto-oncogene/SHP-2 axis.[8] Regarding the passive systemic anaphylaxis, PD-1KO mice exhibited a significantly increased drop in body temperature compared with wild-type (WT) mice (Fig. 1a). Likewise, in the late-phase passive cutaneous anaphylaxis, PD-1KO mice showed a significantly enhanced ear swelling compared with WT mice (Fig. 1b). However, a monoclonal antibody (mAb)-mediated PD-1 receptor blockade did not exacerbate the anaphylactic response (Fig. 1c, d, and Fig. 2).

Monoclonal antibodies targeting the PD-1/programmed death-ligand 1 (PD-L1) pathway or cytotoxic T-lymphocyte antigen 4 have revolutionized the medical oncology. Nonetheless, such a therapeutic approach

inherently runs the risk of evoking immune-related adverse events (irAEs).[9] Previously, we have shown that either genetic deletion of PD-1 or mAb-mediated PD-1 receptor blockade exacerbates allergic contact dermatitis, and we inferred that the PD-1/PD-L1 pathway is a critical regulator of cutaneous irAE (C-irAE).[7] In contrast to the cell-mediated cutaneous immune response, we found that the augmented IgE humoral immune response required the total absence of PD-1, but immunoreceptor engagement was dispensable. This discrepancy may be analogous to the fact that the cytoplasmic PD-1 tail can interact with the SHP-2 phosphatase upon T cell receptor ligation, even in the absence of PD-1 receptor engagement.[2] These lines of evidence and our results further suggest that PD-1 evolved to regulate the adaptive immune response at the effector phase[7] along with other inhibitory immunoreceptors. Our results may also correlate with clinical observations. Although the urticarial rash is the most common form of C-irAE, anaphylaxis, the systemic counterpart of urticaria has never been reported in this context. Therefore, our results could be an important guide for the medical oncology practice in that aggravated IgE-mediated anaphylaxis is due to the total absence of PD-1, but not receptor engagement.

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CONFLICT OF INTEREST

The authors state no conflict of interest.

FIGURE LEGENDS

FIGURE 1. Enhanced passive anaphylaxis in PD-1KO mice, but not in PD-1 mAb treated mice.

(A) Wild-type and PD-1 knockout mice were injected intraperitoneally with 20 μg of anti-dinitrophenol IgE antibody and injected intravenously with 1 mg of DNP-human serum albumin. Rectal temperature was measured for 90 minutes following the challenge. $n = 4$, * $p < 0.05$, two-way ANOVA.

(B) WT and PD-1KO mice were injected intravenously with 2 μg of anti-DNP IgE antibody and painted with 20 μL of 0.3 % 1-fluoro-2,4-dinitrobenzene. Ear thickness was measured 24 hours after the challenge. $n = 6$, * $p < 0.05$, Welch'st -test.

(C) WT mice were administered PD-1 mAb before the induction of passive systemic anaphylaxis. Rectal temperature was measured for 90 minutes following the challenge ($n = 4$).

(D) WT mice were administered PD-1 mAb before the induction of passive cutaneous anaphylaxis. Ear thickness was measured 24 hours after the challenge ($n = 6$).

$\Delta\cdot$ delta, PBS; phosphate-buffered saline, PD-1KO; PD-1 knockout, WT; wild-type, mAb; monoclonal antibody, PD-1; programmed cell death-1, ANOVA; analysis of variance, p ; probability value, n ; number of samples, IgE; immunoglobin E

FIGURE 2. A possible role of intracytoplacmic PD-1 signaling in mast cells.

Multivalent antigen binding causes FcεRI aggregation in mast cells. Cytoplasmic TKs phosphorylate ITAM in the tail of the immunoreceptor resulting in degranulation and mediator/enzyme release.

Normal: ITSM-containing PD-1 cytoplasmic tail recruits SHP-2 and inhibits phosphorylation events.

PD-1KO: The total absence of PD-1 abrogates its interaction with SHP-2 and subsequent dephosphorylation events.

PD-1mAb: Because PD-1 cytoplasmic tail is intact, FcεRI activation could overcome receptor engagement-dependent dephosphorylation events.

Ag; antigen, APC; antigen presentation cell, CM; cell membrane, FcεRI; high-affinity IgE receptor, ITAM; immunoreceptor tyrosine-based activation motif, ITIM; immunoreceptor tyrosine-based inhibitory motif, ITSM; immunoreceptor tyrosine-based switch motif, mAb; monoclonal antibody, PD-1; programmed cell death-1, PD-1KO; PD-1 knockout, PD-L1; programmed death-ligand 1, SHP-2; Src homology 2 domain-containing phosphatase-2, TK; tyrosine-protein kinase.

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