Strength of PD-1 signaling differentially affects T-cell effector functions

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ABSTRACT

Viruses and tumors use a variety of complex techniques to circumvent the immune system and avoid clearance. If these pathogens and tumors are successful in evading the initial immune response, the subsequent immune response directed toward them becomes less and less potent over time. This gradual loss of immune potency is triggered by chronic exposure to antigens and is the result of a distinct immune cell differentiation program called “exhaustion,” which likely evolved to diminish immune-related pathology (1). Within the last several years, much has been done to characterize and determine the phenotypes of exhausted T cells. From these studies, a hierarchy of T-cell dysfunction has emerged in which T cells initially lose their proliferative potential and produce diminished amounts of an immune-stimulating protein known as IL-2. Next, T cells experience diminished cytotoxicity and production of an inflammatory protein known as TNF-α. Finally, T cells lose the ability to produce the immune regulatory protein IFN-γ and ultimately are unable to survive (1). Understanding the molecular mechanisms controlling T-cell exhaustion remains a central goal within the field.

High expression levels of programmed death 1 (PD-1), a cell-surface molecule that, when engaged, blocks immune cell activation, have long been associated with T-cell dysfunction (2), but as yet no studies actually have addressed the extent to which PD-1 expression enforces the exhaustion phenotype. Moreover, with recent discoveries of other negative regulators of T-cell activation such as T-cell immunoglobulin domain and mucin domain 3 (Tim-3), 2B4 (CD244), and lymphocyte-activation gene 3 (LAG-3), as well as T-cell-intrinsic defects that occur with shortening telomeres, the degree to which PD-1 expression contributes to the T-cell exhaustion phenotype is unclear. To answer this question, we developed a physiologic model of T-cell activation that allowed us to modulate PD-1 expression as the only variable (Fig. P1 A) (3). Our studies describe how variations in PD-1 expression affect T-cell function and provide insight into T-cell exhaustion and therapeutics that target PD-1.

In particular, we found that the introduction of high levels of PD-1 into highly functional primary human CD8 T cells, immune cells whose main job is to kill infected cells or tumors, resulted in severe inhibition of T-cell function. This finding indicates that PD-1 expression alone is sufficient to mediate the T-cell exhaustion phenotype. Moreover, using a peptide-counting approach that quantifies the number of cognate peptide/MHC complexes (pMHC) required to stimulate a T cell, we observed that PD-1 engagement made T cells less sensitive to T-cell receptor (TCR)-mediated signals. Although 10 pMHC complexes were required to induce maximal T-cell activation measured by quantifying changes in intracellular Ca2+ concentration (Ca2+ flux) in the absence interactions between PD-1 and ligand 1 (PD-L1), we observed that 30 clustered pMHC complexes were required in the presence of PD-1 signaling (Fig. P1 B). Last, we found that T-cell functions are differentially affected by PD-1 ligation. We observed that proliferation, as well as the production of IL-2 and TNF-α, are highly sensitive to PD-1 signals. In contrast, cytotoxicity and the production of IFN-γ and macrophage inflammatory protein 1 beta (MIP-1β) require far stronger PD-1-mediated signals to be inhibited. These data are summarized in Fig. P1 C. Together, these data suggest that PD-1 ligation is likely to be a main culprit in the inability of exhausted T cells to produce IL-2 and proliferation, whereas other negative regulators, alone or in collaboration with PD-1, are responsible for the inability of exhausted T cells to kill targets efficiently and produce IFN-γ.


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the recent clinical success of anti–PD-1 Ab therapy alone or in combination with anti–cytotoxic T-lymphocyte antigen 4 (CTLA-4) Ab therapies (4), the studies performed here will elucidate how these therapies result in impressive and durable tumor regressions.

By performing quantitative assays to measure how PD-1 signaling affects T-cell function, we provide a key resource for understanding the effects of PD-1 blockade relevant to therapeutic applications (5). Having established a hierarchy of the T-cell functions most affected by PD-1 engagement, we provide a tool for understanding how PD-1 blockade behaves in vivo. Our data show how negative regulators such as PD-1 control T-cell activation, and these findings provide a platform to appreciate how agents targeting various negative regulators of T cells might best be paired to improve T-cell responses to chronic antigen exposure.