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Replenish the source within

Rescuing tumor-infiltrating lymphocytes by double checkpoint blockade

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We have recently reported that the PD-1 and CTLA4 signaling pathways are active in both effector and regulatory T cells, causing profound immune dysfunctions in the tumor microenvironment. In line with this notion, the dual blockade of PD-1- and CTLA4-conveyed signals may exert robust therapeutic effects. Here, we discuss the mechanisms possibly underlying such a synergic interaction.

Our group and others have revealed that tumor-infiltrating T lymphocytes (TILs) with antineoplastic activity exists in cancer patients, yet are often insufficient to inhibit tumor growth.[1] The increased expression of immunosuppressive molecules is one major mechanism whereby tumors evade immune response. One of these molecules is programmed death cell death 1 (PDCD1) ligand 1 (CD274, best known as PD-L1), whose intratumoral expression levels are inversely correlated with survival in patient affected by several malignancies.[2] In addition, TILs manifest increased expression levels of other immunosuppressive molecules, such as cytotoxic T-lymphocyte-associated protein 4 (CTLA4).[3] However, the precise immunosuppressive mechanisms activated by the simultaneous engagement of multiple of these receptors (as opposed to the activation of individual immunosuppressive pathways), have not yet been delineated.

We addressed this question by assessing the expression of PDCD1 (best known as PD-1), CTLA4 and their ligands in murine models of colon and ovarian carcinoma.[4] We found that the blockade of PD-1 in vivo activates T cells to mediate antineoplastic effects, a phenomenon that is greatly exacerbated by the addition of anti-CTLA4 antibodies. Moreover, we observed that a significant percentage of tumor-infiltrating $CD8^+$ T cells expressed high levels of PD-1 ($PD\text{-}1^{hi}$ cells), and that only a fraction of these cells also expressed CTLA4. Conversely, very few $CD8^+$ T cells were found to express CTLA4 but not PD-1. Importantly, most among the tumor-associated antigen (TAA)-specific $CD8^+$ T cells belonged to the double positive ($PD\text{-}1^{hi}CTLA4^+$) population. Of note, such double positive cells appear to be functionally impaired (as demonstrated upon stimulation with a TAA-derived peptide), whereas $PD\text{-}1^{hi}$ $CD8^+$ T cells maintained some function ex vivo, including a proliferative response to antigenic stimulation. These observations indicate that tumor-infiltrating $PD\text{-}1^{hi}$ $CD8^+$ T cells are not entirely exhausted, while their $PD\text{-}1^{hi}CTLA4^+$ counterparts exhibit a severely exhausted or anergic phenotype.[5]

The mechanisms through which blocking immunological checkpoints rescues immune function in tumors is the subject of active investigation. The blockade of PD-1/PD-L1 interactions exerts direct stimulatory effects on $CD8^+$ T cells. In addition, the blockade of CTLA4 and PD-1 can also activate T cells upon the attenuation of immunoregulatory circuitries, such as those mediate by $FOXP3^+CD4^+$ regulatory T cells (Tregs), and there is evidence of a cooperative interaction between these T cell-intrinsic and extrinsic effects (**Fig. 1**). For example, the blockade of CTLA4 on both Tregs and effector T cells (Teffs) enhances antitumor immunity more efficiently than the inhibition of the signaling pathways on either cell population alone. Moreover, mice that completely lack CTLA4 expression develop more aggressive neoplasms than animals in which the CTLA4 deficiency is limited to Tregs.[3] A recent study has revealed that the PD-1 pathway also promotes the function of Tregs,[6] and the blockade of PD-L1 was found to enhance the activity of Teffs against melanoma by attenuating their inhibition by Tregs.[7] In our models, tumor-infiltrating Tregs accumulated in parallel with the accumulation of dysfunctional $PD\text{-}1^+CTLA4^+$ T cells. In this setting, the anti-CTLA4 antibody 9D9, an IgG2b, did not deplete Tregs or impair their function in vivo, but promoted the proliferation of Teffs. Importantly, the blockade of PD-1 in

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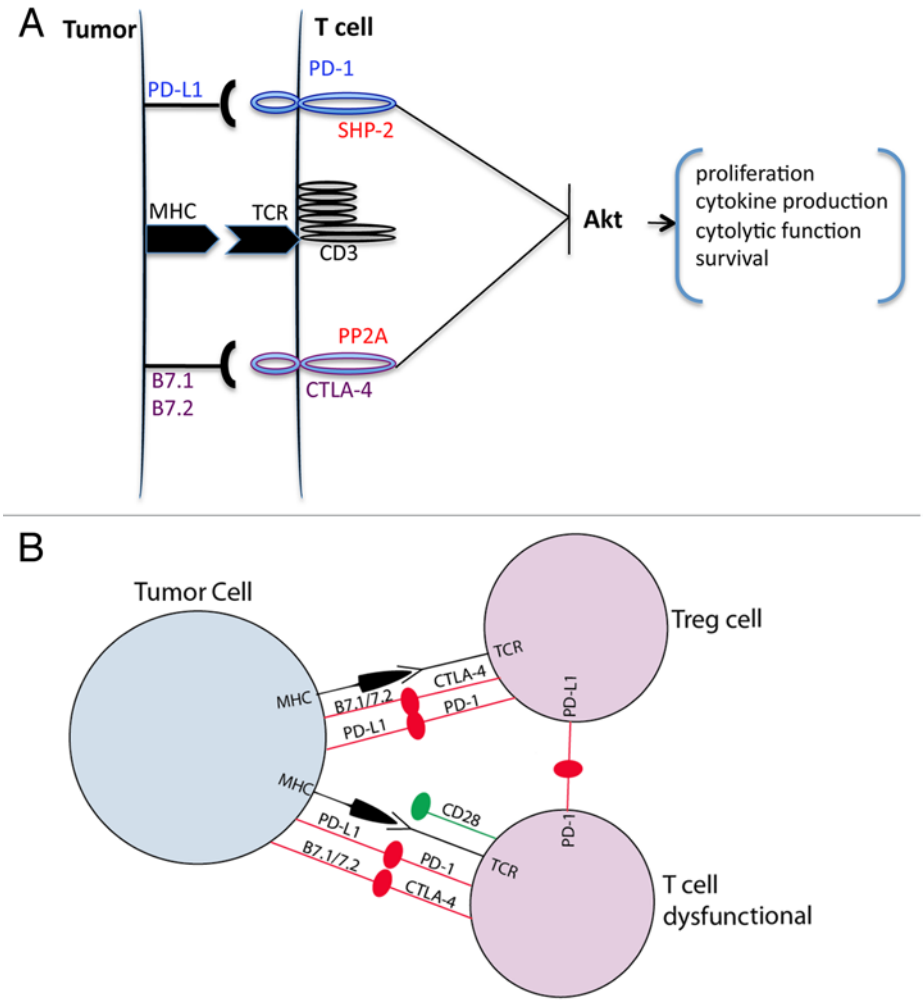

Figure 1. Blocking PD-1- and CTLA4-dependent immunological checkpoints synergistically enhances TCR signaling in both effector and regulatory T cells. (**A**) AKT phosphorylation is blocked by both PD-1- and CTLA4-transmitted signals, yet through different mechanisms. Indeed, while PD-1 recruits the tyrosine phosphatase SHP2, CTLA4 operates through the serine/threonine phosphatase PP2A.[5] (**B**) Antitumor immune responses are inhibited following the upregulation of PD-L1 by malignant cells as well as the expression of CTLA4, PD-L1 and PD-1 by regulatory T cells (Tregs). PD-L1 on Tregs can indeed transmit direct immunosuppressive signals upon interaction with PD-1 on $CD8^+$ T cells. Blocking PD-1-, PD-L1-, and CTLA4-dependent regulatory signals boost TCR and CD80/CD86 (B7.1/B7.2) signaling, hence reversing the dysfunction that generally affects T cells in the tumor microenvironment.

vivo had a moderate effect on Treg-mediated immunosuppression. These observations offer an additional explanation for the positive interaction between PD-1 and CTLA4 blockade. Indeed, the net effect of Teff inhibition appears to depend on the functional outcome of multiple receptor-ligand interactions not only between Teffs and antigen-presenting and/or target cells, but also on Tregs. While the blockade of PD-1 or CTLA4 restores the antitumor activity of dysfunctional T cells, the simultaneous inhibition of PD-1 and CTLA4 also limits the activity of Tregs. It has recently been reported that anti-CTLA4 IgG2a antibodies that promote antibody-dependent cell-mediated cytotoxicity (ADCC) robustly deplete tumor-infiltrating Tregs and are far more effective than other IgG isotypes that exhibit similar neutralizing activity but stimulate weak or no ADCC.[8] Combining such Treg-depleting antibodies with PD-1 blockade is likely to exert superior antitumor effects.

In recent clinical studies, the intravenous administration of anti-PD-1 or anti-CTLA4 monoclonal antibodies to patients with advanced solid tumors has been associated with tolerable adverse events and promising clinical responses.[9] This validates the translational relevance of a large amount of preclinical investigation and generates a great degree of enthusiasm. However, it is important to further understand how we can modulate the PD-1 and CTLA4 signaling pathways to boost TAA-specific T cells while avoiding systemic T-cell activation. Our study supports the notion of combining the dual blockade of PD-1 and CTLA4 signaling with whole tumor cell vaccines. Indeed, although vaccination alone was ineffective in mice as it had been in the clinic,[10] such a combinatorial regimen resulted in a robust therapeutic interaction. In spite of the technical and managerial challenges posed by autologous whole tumor cell vaccines,

such a combination may open the door to effectively targeting poorly immunogenic and highly aggressive cancers.

Taken together, our findings show that the expression of PD-1 and CTLA4 by TILs correlate with the degree of T-cell dysfunction in the tumor microenvironment, and that PD-1^{hi}CTLA4^{+} T cells comprise many TAA-specific T cells, strengthening the rationale for simultaneously targeting these immunosuppressive pathways. However, multiple questions remain unanswered. What are the precise molecular mechanisms mediating the positive interaction between the simultaneous blockade of PD-1 and CTLA4 signaling? How are the T cell-intrinsic and extrinsic effects of PD-1 and CTLA4 linked to each other? And which other maneuvers are critical to condition the tumor microenvironment so to efficiently promote tumor rejection? Future studies will have to address these important issues.

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Disclosure of Potential Conflicts of Interest

GJ Freeman draws royalties from patents regarding PD-1.

References

1. Zhang L, Conejo-Garcia JR, Katsaros D, Gimotty PA, Massobrio M, Regnani G, Makrigiannakis A, Gray H, Schlienger K, Liebman MN, et al. Intratumoral T cells, recurrence, and survival in epithelial ovarian cancer. N Engl J Med 2003; 348:203-13; PMID:12529460; http://dx.doi.org/10.1056/NEJMoa020177
2. Hamanishi J, Mandai M, Iwasaki M, Okazaki T, Tanaka Y, Yamaguchi K, Higuchi T, Yagi H, Takakura K, Minato N, et al. Programmed cell death 1 ligand 1 and tumor-infiltrating CD8+ T lymphocytes are prognostic factors of human ovarian cancer. Proc Natl Acad Sci USA 2007; 104:3360-5; PMID:17360651; http://dx.doi.org/10.1073/pnas.0611533104
3. Curran MA, Montalvo W, Yagita H, Allison JP. PD-1 and CTLA-4 combination blockade expands infiltrating T cells and reduces regulatory T and myeloid cells within B16 melanoma tumors. Proc Natl Acad Sci U S A 2010; 107:4275-80; PMID:20160101; http://dx.doi.org/10.1073/pnas.0915174107
4. Duraiswamy J, Kaluza KM, Freeman GJ, Coukos G. Dual blockade of PD-1 and CTLA-4 combined with tumor vaccine effectively restores tumor rejection function in tumors. Cancer Res 2013; 73:3591-603; PMID:23633484; http://dx.doi.org/10.1158/0008-5472.CAN-12-4100
5. Wei F, Zhong S, Ma Z, Kong H, Medvec A, Ahmed R, Freeman GJ, Krogsgaard M, Riley JL. Strength of PD-1 signaling differentially affects T-cell effector functions. Proc Natl Acad Sci U S A 2013; 110:E2480-9; PMID:23610399; http://dx.doi.org/10.1073/pnas.1305394110
6. Francisco LM, Salinas VH, Brown KE, Vanguri VK, Freeman GJ, Kuchroo VK, Sharpe AH. PD-L1 regulates the development, maintenance, and function of induced regulatory T cells. J Exp Med 2009; 206:3015-29; PMID:20008522; http://dx.doi.org/10.1084/jem.20090847
7. Wang W, Lau R, Yu D, Zhu W, Korman A, Weber J. PD1 blockade reverses the suppression of melanoma antigen-specific CTL by CD4+ CD25(Hi) regulatory T cells. Int Immunol 2009; 21:1065-77; PMID:19651643; http://dx.doi.org/10.1093/intimm/dxp072
8. Selby MJ, Engelhardt JJ, Quigley M, Henning KA, Chen T, Srinivasan M, Korman AJ. Anti-CTLA-4 Antibodies of IgG2a Isotype Enhance Antitumor Activity through Reduction of Intratumoral Regulatory T Cells. Cancer Immunol Res 2013; 1:32-42; http://dx.doi.org/10.1158/2326-6066.CIR-13-0013
9. Wolchok JD, Kluger H, Callahan MK, Postow MA, Rizvi NA, Lesokhin AM, Segal NH, Ariyan CE, Gordon RA, Reed K, et al. Nivolumab plus ipilimumab in advanced melanoma. N Engl J Med 2013; 369:122-33; http://dx.doi.org/10.1056/NEJMoa1302369; PMID:23724867
10. Dranoff G. GM-CSF-secreting melanoma vaccines. Oncogene 2003; 22:3188-92; PMID:12789295; http://dx.doi.org/10.1038/sj.onc.1206459