PD-1 and BTLA and CD8+ T-cell “exhaustion” in cancer
“Exercising” an alternative viewpoint

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Keywords: exhaustion, cancer, PD-1, BTLA, and other co-inhibitory molecules on T cells from cancer patients has become an accepted signature for a state called T-cell “exhaustion” that has emerged almost as dogma in the field. However, here we propose that in some cases this “exhausted” T-cell phenotype may instead be an indicator of T cells that are in a more heightened state of T-cell activation more susceptible to negative regulation rather than being “exhausted.” This alternative interpretation fits in line with the view that CD8+ T-cell activation in cancer results from a continuum of signals regulating their differentiation towards potent effector cells.

Biomarkers are especially attractive in immunology as a way of identifying a subpopulation of cells based on their differentiation, function, or activation state. One way of describing activation, as proposed in models of viral infection (e.g., by HIV or the lymphocytic choriomeningitis virus LCMV) is the concept of “exhaustion.” Exhausted T cells have been defined as a pool of dysfunctional cells resulting from chronic antigen stimulation, which are unable to respond further (cell progressively lose their ability to proliferate, secrete cytokines and exert cytotoxic functions), even after resting.6 Negative costimulatory markers associated with exhaustion include: PD-1, TIM-3, LAG-3 and the recently described B- and T-lymphocyte attenuator (BTLA).2,3 Such exhaustion markers have also been utilized to describe the state of tumor-infiltrating lymphocytes (TILs) and tumor-specific T cells in the peripheral blood.6,3 However, are these cells really exhausted? Is exhaustion conceptually the way we should view these cells?

This question goes to the heart of how we view CD8+ T-cell activation and differentiation as well as of how these processes can be altered in cancer. High expression of PD-1 and other co-inhibitory molecules have been described as markers of dysfunctional and exhausted T cells following repeated antigenic stimulation, especially in cells isolated from the tumor microenvironment.4,5 However, are these cells really exhausted? Is exhaustion conceptually the way we should view these cells?

This question goes to the heart of how we view CD8+ T-cell activation and differentiation as well as of how these processes can be altered in cancer. High expression of PD-1 and other co-inhibitory molecules have been described as markers of dysfunctional and exhausted T cells following repeated antigenic stimulation, especially in cells isolated from the tumor microenvironment. However, many of these studies do not analyze additional phenotypic and functional attributes of these T cells that may be critical, especially their memory and differentiation status (i.e., the pathway toward fully differentiated cytotoxic T lymphocytes, CTLs). Furthermore, in vivo blockade of PD-1 and TIM-3 has been shown to restore antiviral and antitumor responses and T cells placed in culture ex vivo rapidly regain their full activity.6-8 In the field of tumor immunology, an alternative view of exhaustion is that of a state in which T cells are highly activated, yet incompletely differentiated, hence lacking the expected effector functions of cytokine secretion and potent CTL activity. Thus, these cells are really effector-memory T cells in a heightened state of activation resulting from repeated stimulation. The increased expression of co-inhibitory molecules could be interpreted as a normal immune regulatory mechanism preventing these cells from becoming more activated and hence potentially harmful. Recent work by Duriwal et al. supports this
view. In an elegant gene expression analysis on sorted CD8+PD-1+ T cells from healthy donors, they found that CD8+PD-1+ T cells have a gene signature that does not overlap with that of “exhausted” T cells from HIV-infected patients, but rather with that of previously activated effector-memory cells that would normally be on their way to differentiating into effector cells. Thus, it is possible that PD-1+CD8+ T cells in cancer patients may not be necessarily exhausted and be simply activated effector-memory cells that persist without further differentiating; a state in which they have a lower proliferative potential and susceptible to more negative regulation by the immune system.

Given these premises, it would make sense that CD8+ T cells isolated from tumors (which indeed are previously primed effector or effector-memory cells) exhibit an elevated state of activation due to repeated antigenic stimulation and/or the pro-inflammatory tumor micro-environment. These cells may be trying to differentiate into effector cells but are unable to do so due to a potential lack of proper co-stimulation or to the unfavorable cytokine milieu. Therefore, these cells would exhibit elevated levels of PD-1 expression as well as of other activation/co-inhibition markers like TIM-3 and LAG-3, but would not downregulate BTLA (which normally happens when T cells differentiate). This view is...
supported by work in melanoma TILs showing that tumor antigen-specific CD8+ T cells are enriched in the PD-1+ population. These seemingly “exhausted” CD8+ T cells are highly susceptible to negative regulation because they are trying to do what they are supposed to do—differentiate into effector cells. However, while the underlying reasons remain unclear, these cells are partially differentiated, they have lost some of their proliferative potential.

Another possible interpretation explaining the presence of seemingly exhausted CD8+ T cells in cancer is that PD-1+ cells might have recently been stimulated by PD-1 ligands (PD-L1 or PD-L2) in vivo, a scenario that appears very likely in cancer patients. This may occur frequently, especially when PD-1 becomes constitutively expressed following the hypomethylation of its locus in the genome. Thus, signaling via PD-1 may persist over protracted periods of time, even after cell isolation, resulting in the inhibition of T-cell activation and expansion after ex vivo re-stimulation. (Fig. 1). This can be mistakenly interpreted as T-cell exhaustion, a term implying a fundamental shift in the differentiation program of T cells, rather than an ongoing inhibitory signal that has persisted through isolation and sorting procedures. This view is supported by the fact that in many cases, the isolated T cells rapidly regain their ability to divide and perform effector functions after a few days or even a few hours in culture.

We and others have demonstrated that CD8+ TILs can be induced to proliferate ex vivo despite high levels of PD-1, BTLA, TIM-3 and other exhaustion-associated markers and that these TILs can mediate potent antitumor responses upon adoptive transfer into patients. Thus, either exhaustion does not exist in practice or it is a normal consequence of an incomplete differentiation program seen in viral-specific T cells.

Additional work by the Lausanne group compared the expression and function of BTLA in CD8+ T cells (from MART-1 peptide-vaccinated melanoma patients) at different stages of differentiation to virus-specific T cells at similar stages of differentiation. They found that BTLA was not upregulated by MART-1-specific CD8+ T cells, but rather not downregulated as a result of the normal T cell differentiation program seen in viral-specific T cells. Furthermore, when the same authors looked at another cohort of patients vaccinated with the MART-1 peptide, this time in together with CpG oligonucleotides (to activate innate immune and stimulate Type I IFN production and maturation of dendritic cells, DCs), antigen-specific CD8+ T cells were found to often become BTLA-negative (presumably due to BTLA downregulation) and acquire more potent antitumor effector cell properties. This supports the view that primed MART-1-specific CD8+ T cells expressing BTLA from the first cohort were not exhausted but rather that CD8+ T-cell differentiation was defective. Thus, the co-expression of BTLA and PD-1 may simply mark highly activated T cells not proceeding through their cell cycle course of differentiation toward exhausted patients. However, as implicated by the results of Speiser’s group, manipulating the system with activators of the innate immune system (e.g., CpG) may restore the CD8+ T-cell differentiation program (Fig. 1, Model 2).

Thus, CD8+ T cells from tumors and the periphery expressing PD-1, BTLA and other co-inhibitory molecules can be viewed as highly activated T cells that can be modulated through immunotherapy, and not as “exhausted” cells. This concept proposes “the antitumor immunity glass” as half-full, not half-empty. The decreased ex vivo proliferative capacity of these cells is arguably a normal consequence of an incomplete differentiation toward effector cells and not of an actual exhaustion. Therefore, it may be counterproductive to remove these supposedly exhausted cells as a means of improving antitumor immunity. These cells are simply doing what they have evolved to do in these settings: not indiscriminately proliferate, but balance proliferation with a differentiation program seen in viral-specific T cells.

In summary, we suggest that—as tumor immunologists—we keep an open mind towards our conceptual view of T-cell exhaustion when describing tumor antigen-specific T cells isolated from cancer patients expressing PD-1, BTLA, and other co-inhibitory molecules toward a view that keeps options open as to the true nature of the cells. More work needs to be performed to clearly define how T cells (especially CD8+ CTLs) normally differentiate (or don’t differentiate) in...
cancer patients. This will elucidate whether the supposedly exhausted phenotype that many are describing indeed represents a state in which T cells exhibit a high activation status, are more prone to negative regulation and have not completely differentiated (Fig. 1). We surmise that blocking PD-1 and other inhibitory molecules expressed on these so-called “exhausted,” previously tumor antigen-primed, CD8+ T cells, or activating innate immune signaling (e.g., with CpG or other TLR agonists), might rapidly unlock this blocked CTL differentiation pathway in cancer patients.

Grant Support
This work was supported by National Cancer Institute (NCI) grant RO1-CA111999 to L.R., by The University of Texas MD Anderson Cancer Center Support Grant (P30-CA16672), and the Mulva Foundation.

References
1. Wherry EJ, Baltimore DF, Naresh-Kumar K, van der Most R, Ahmed R. Viral persistence above CD8 T-cell immunosurveillance and tissue distribution affect results in distinct stages of functional impairment. J Virol 2003; 77:4911-27. PMID:12667877; http://dx.doi.org/10.1128/JVI.77.7.4911-4927.2003
2. Youngblood B, Wherry EJ, Ahmed R. Acquired immunodominance in functional and exhausted transgenic CD8+ T cells. Curr Opin Immunol 2010; 22:70-7. PMID:20331844; http://dx.doi.org/10.1016/j.coi.2010.08.007.
3. Angelosanto JM, Wherry EJ. Transcription factor regulation of CD8+ T-cell memory and exhaustion. Immunol Rev 2010; 236:167-75. PMID:20256814; http://dx.doi.org/10.1111/j.1600-065X.2010.01297.x.
4. Ahmadzadeh M, Johnson LA, Honess DJ, Wunderlich JR, Dudley ME, White DE, et al. Tumor antigen-specific CD8 T cells infiltrating the tumor express high levels of PD-1 and are functionally impaired. Blood 2009; 114:1537-44. PMID:19566617; http://dx.doi.org/10.1182/blood-2008-12-194237.
5. Duraiswamy J, Ibegbu CC, Masopust D, Miller JD, Akondy RS, Akbari OX, et al. Chronic virus infection enforces demethylation of the locus that encodes PD-1. Science 2011; 331:400-12. PMID:21943489; http://dx.doi.org/10.1126/science.1195478.
6. Barber DL, Wherry EJ, Masopust D, Brdicka R, Ahmed R. Viral persistence alters CD8 T-cell responses in heavily pretreated patients with metastatic disease. Int J Cancer 2012; 130:2327-36. PMID:22038851; http://dx.doi.org/10.1002/ijc.26272.
7. Duraiswamy J, Durand RA, Young GD, Xu H, Sharpe AH, et al. Restoring function in exhausted virus-specific CD8 T cells. Curr Opin HIV AIDS 2011; 6:91-8. PMID:21555851; http://dx.doi.org/10.1097/COH.0b013e32834ddcf2.
8. Topalian SL, Drake CG, Pardoll DM. Targeting the PD-1/PD-L1 pathway to activate antitumor immunity. Curr Opin Immunol 2012; 24:207-12. PMID:22320695; http://dx.doi.org/10.1016/j.coi.2011.12.005.
9. Dummer R, Buzaid CC, Maespert D, Miller JS, Ambudkar V, Dole O, et al. Phenotype, function, and gene expression profile of programmed death-1 (PD-1) positive CD8 T cells in healthy human adults. J Immunol 2011; 186:4910-12. PMID:21383245; http://dx.doi.org/10.4049/jimmunol.1000783.
10. Derré L, Brade JP, Jardieu C, Parent S, Birdell D, Benoist P, et al. RTLA mediated inhibition of human tumor-specific CD8+ T cells that can be partially reversibly induced. J Clin Invest 2010; 120:1157-67. PMID:20008811; http://dx.doi.org/10.1172/JCI46102.
11. Youngblood B, Oestreich KJ, Ha SJ, Duraiswamy J, and autoimmunity in patients after clonal repopulation with autologous tumor-infiltrating lymphocytes for metastatic melanoma: current status and future outlook. Cancer J 2012; 18:160-75. PMID:22453018; http://dx.doi.org/10.1097/PPO.0b013e31824d4465.
12. Dudley ME, Wunderlich JR, Robbins PF, Yang JC, Hughes MS, Phan GQ, et al. Durable complete responses in heavily pretreated patients with metastatic melanoma using autologous tumor-infiltrating lymphocytes for metastatic melanoma: current status and future outlook. Science 2002; 298:850-4. PMID:12242449; http://dx.doi.org/10.1126/science.1079809.
13. Topalian SL, Drake CG, Pardoll DM. Targeting the PD-1/PD-L1 pathway to activate antitumor immunity. Curr Opin Immunol 2012; 24:207-12. PMID:22320695; http://dx.doi.org/10.1016/j.coi.2011.12.005.
14. Wu RFM-A, Forget M-A, Chacon J, Bernatchez C, Sander C, et al. CD8(+) T cells specific for tumor antigens can be reinvigorated by the tumor microenvironment through upregulation of the inhibitory receptors RTLA and PD-1. Cancer Res 2012; 72:987-95. PMID:22038875; http://dx.doi.org/10.1158/0008-5472.CAN-11-3827.
15. Dudley ME, Young GD, Xu H, Zulewski H, Sander C, et al. CD8(+) T cells specific for tumor antigens can be reinvigorated by the tumor microenvironment through upregulation of the inhibitory receptors RTLA and PD-1. Cancer Res 2012; 72:987-95. PMID:22038875; http://dx.doi.org/10.1158/0008-5472.CAN-11-3827.
16. Krönig H, Julia Falchner K, Odendahl M, Brackertz B, Perna F, et al. Chronic virus infection microenvironment through upregulation of the inhibitory receptors RTLA and PD-1. Cancer Res 2012; 72:987-95. PMID:22038875; http://dx.doi.org/10.1158/0008-5472.CAN-11-3827.
17. Topalian SL, Drake CG, Pardoll DM. Targeting the PD-1/PD-L1 pathway to activate antitumor immunity. Curr Opin Immunol 2012; 24:207-12. PMID:22320695; http://dx.doi.org/10.1016/j.coi.2011.12.005.
18. Dudley ME, Wunderlich JR, Robbins PF, Yang JC, Hughes MS, Phan GQ, et al. Durable complete responses in heavily pretreated patients with metastatic melanoma using autologous tumor-infiltrating lymphocytes for metastatic melanoma: current status and future outlook. Cancer J 2012; 18:160-75. PMID:22453018; http://dx.doi.org/10.1097/PPO.0b013e31824d4465.
19. Baitsch L, Legat A, Balle L, Faustino Muratou GA, Bottger P, Baitsch W, et al. Expression of inhibitory receptors by human CD8 T-cells depending on differentiation, antigen-specificity and anatomical localization. PLoS One 2012; 7:e30852. PMID:22347406; http://dx.doi.org/10.1371/journal.pone.0030852.
20. Sakthivel P, Gereke M, Bruder D. Therapeutic intervention in cancer and chronic viral infections: antibody mediated manipulation of PD-1/PDL1 interaction. Rev Recent Clin Trials 2012; 7:130-25. PMID:22025178; http://dx.doi.org/10.2174/157488712799363262.
21. Sakthivel P, Gereke M, Bruder D. Therapeutic intervention in cancer and chronic viral infections: antibody mediated manipulation of PD-1/PDL1 interaction. Rev Recent Clin Trials 2012; 7:130-25. PMID:22025178; http://dx.doi.org/10.2174/157488712799363262.