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New insights on the role of CD8+CD57+ T-cells in cancer

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Abbreviations: CTL, cytotoxic T lymphocytes; TCR, T cell receptor; TIL, tumor-infiltrating lymphocytes; ACT, adoptive T-cell therapy; PBMC, peripheral blood mononuclear cells; Perf, perforin; GB, granzyme B; HNK-1, human natural killer-1; T_N, naïve T cells; T_{EM}, effector-memory T cells; T_{TDE}, terminally-differentiated effector cells

Incomplete differentiation of CD8+ cytotoxic T-lymphocytes (CTLs) in the tumor microenvironment is associated with cancer progression. We describe a new type of tumor-infiltrating CD8+CD57+ T cell in cancer with hybrid phenotypic and functional properties of both an early effector-memory cell and a terminally-differentiated effector cell. These cells behave as incompletely-differentiated CTLs.

Melanomas are considered to be one of the most immunogenic cancers and are frequently infiltrated with CD8+ and CD4+ T lymphocytes specific for major tumor antigens. CD8+ cytotoxic T lymphocytes (CTL) mediate antigen-specific lysis of tumor cells through cytolytic granule proteins, such as granzymes, granulysin and perforin. Previously, other investigators have shown a lack of complete CTL differentiation in solid tumors.[1] However, the exact stage of differentiation affected was unclear. We recently reported in *Clinical Cancer Research* a novel subset of CD8+ tumor-infiltrating lymphocytes (TILs) that co-expressed early effector-memory markers (CD27 and CD28) and a marker for end-stage, senescent T cells (CD57).[2]

CD28 and CD27 are markers of early CD8+ effector-memory T (T_{EM}) cells. Based on studies on virus-specific T cells in humans, it was postulated that CD8+ T_{EM} cells differentiate in a linear pathway from CD28+CD27+ (early-differentiated) to CD28-CD27+ (intermediate-differentiated) to CD28-CD27- (late-differentiated).[3] As CD8+ T_{EM} transitions to end-stage effector (T_{EFF}), the loss of CD28 and gain of CD57 is an immunological characteristic of humans and non-human primates, but not of mice.[3] CD57 is a marker on highly differentiated CD8+CD27-CD28- T cells needed to control CMV and other endemic viruses in humans.[3,4] CD57 was also proposed as a marker of end-stage, senescent CD8+ T cells in HIV patients exhibiting highly cytotoxic potential.[3,5] However, in HIV progressors, a failure to coordinately downregulate CD27 and upregulate CD57 resulted in an accumulation of an unusual subset of HIV-specific CD8+CD27+CD57+ cells.[4] CD8+CD27+CD57+ T lymphocytes have also been observed in the peripheral blood of melanoma patients after vaccination with gp100 tumor antigen.[6]

We set out to determine the role of CD8+CD57+ T cells in the melanoma. By performing flow cytometry staining on TIL isolated from 44 metastatic tumors, we found that the CD8+CD57+ subset was 16.2 ± 3.5% of the total tumor-infiltrating CD8+ T cells. The vast majority of these CD8+ T cells were also "locked" in the T_{EM} stage and, on average, > 20% of the CD8+CD57+ subset co-expressed CD27 and CD28. These T cells were GB^{hi} but $Perf^{lo/-}$, and they recognized melanoma tumor antigens, MART-1 and gp100 in HLA-A2+ patients. In contrast, only a few (< 5%) of the CD8+CD57+ T cells in the PBMC of the same melanoma patients co-expressed CD27 and CD28, but they were GB^{hi} and $Perf^{hi}$. We also found a similar population in pleural effusions from metastatic breast cancer. Notably, this TIL subset was different from the Foxp3+ CD8+ early effector TILs, which did not express CD57, as reported earlier in reference 7.

Whether CD8+CD57+ T cells are really "senescent" has become a controversial issue.[3] We addressed this issue in the CD8+CD27+CD57+ TIL subset and found that they indeed proliferated in response to IL-2. We then purified this subset from IL-2-cultured TILs by cell sorting. Interestingly, we found that the CD27+CD57+ TILs were able to proliferate and produce high levels of IFNγ upon TCR stimulation, which was inconsistent with CD57 as a general marker for T-cell senescence. We also found that PD-1 expression was higher in the CD27+CD57+ subset compared with the CD27+CD57- subset. Since CD8+ T cells naturally express PD-1 as a result of TCR activation, where it plays a regulatory mechanism to prevent over-reactivity,[3] it

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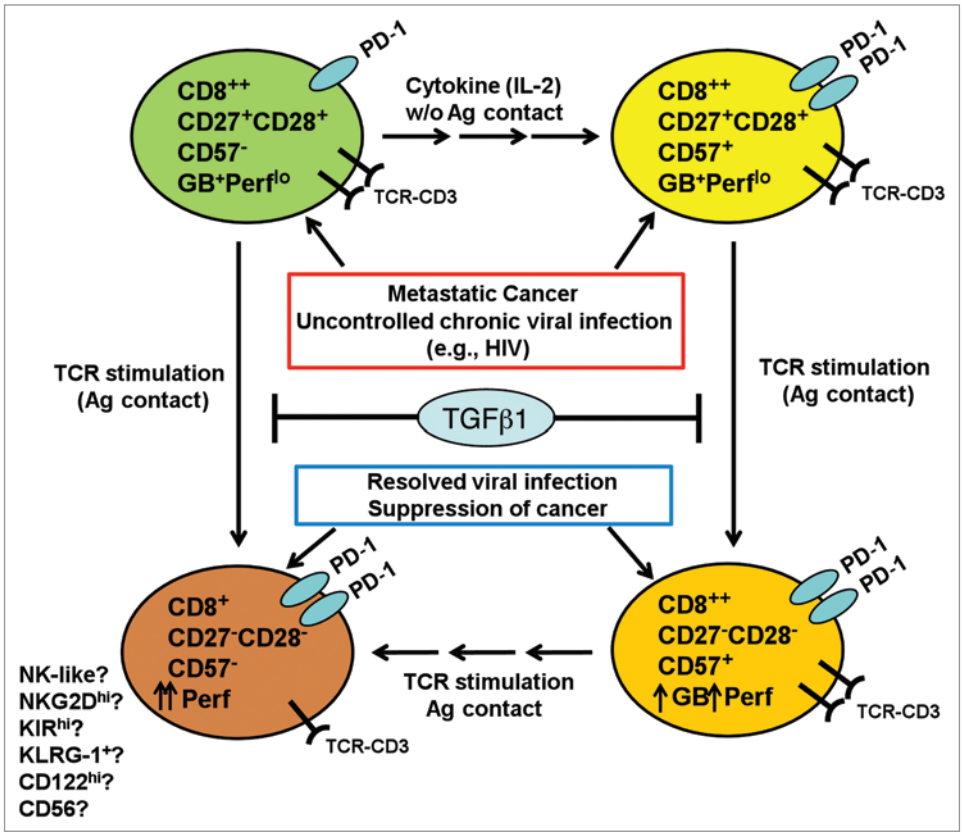

Figure 1. Differentiation pathway of the tumor-infiltrating CD8$^+$ T cells in metastatic cancer. In situations where CD8$^+$ T cells encounter persistent, chronic antigenic stimulation such as metastatic cancer or uncontrolled chronic viral infections, CD8$^+$ effector-memory T (T_{EM}) cells fail to coordinate downregulation of CD27 with upregulation of an end-stage CTL marker, CD57 and acquire a more cytolytic phenotype. Thus T_{EM} fail to transition from a granzyme B (GB$^+$) perforin^{lo} (Perf^{lo}) cells into Perf^{hi}, highly cytotoxic end-stage CTL. This resulted in accumulation of CD8$^+$ T cells at a transitional stage where markers for early T_{EM} (CD27, CD28) are co-expressed with CD57, even though the cells remain Perf^{lo}. We also found that TGFβ1, an immunosuppressive cytokine frequently found in the microenvironment of metastatic cancer, could also contribute to the arrested differentiation and accumulation of CD27$^+$CD57$^-$ precursor T cells and CD27$^+$CD57$^+$ T cells. We found that PD-1 was expressed more in the CD27$^+$CD57$^+$ T cells, which implied that they may exhibit a higher level of effector activity. When these tumor-infiltrating lymphocytes (TIL) are expanded with IL-2, a minor fraction (~30%) of CD27$^+$CD57$^-$ subset differentiated into CD27$^+$CD57$^+$ T cells. After TCR stimulation ex vivo, the CD27$^+$CD28$^+$CD57$^-$ subset directly differentiated to become CD27$^-$CD57$^-$ T cells. On the other hand, CD27$^+$CD57$^+$ differentiated to become CD27$^-$CD57$^+$, and in some patients, CD27$^-$CD57$^-$. These phenotypic changes were accompanied by increased perforin expression and acquisition of potent cytotoxicity against tumor cells. We think that CD57 is not a marker for T-cell senescence, but rather marks highly differentiated T cells that are in the process of transitioning into a truly end-stage, effector CTL. Currently it is not known which set of markers define truly senescent, end-stage, highly cytolytic CTL. We also propose that ultimately, both the CD8$^+$CD57$^+$ and CD8$^+$CD57$^-$ subsets may ultimately differentiate into a subset of CD8$^+$CD27$^-$CD28$^-$CD57$^-$ cells that may be more NK-like, expressing higher levels of CD122, KLRG1, NKG2D and other NK receptors.

is possible that the CD8$^+$CD27$^+$CD57$^+$ subset are more highly activated T cells in the tumor microenvironment.

Building on our observation that CD8$^+$CD57$^+$ TILs were non-senescent, we hypothesized that these T cells were in a transition state and could be induced to differentiate further to cytotoxic end-stage effectors. Indeed, we found that the sorted CD8$^+$CD27$^+$CD57$^+$ TILs, which were Perf^{lo} and poorly cytotoxic, could differentiate into a Perf^{hi}, highly cytotoxic CD27$^-$CD57$^+$ or CD27$^-$CD57$^-$ subset after TCR stimulation (**Fig. 1**). IL-2 treatment alone induced a minor fraction of CD27$^+$ precursor cells to become CD27$^+$CD57$^+$, which suggested that IL-2 was sufficient to expand the CD27$^+$CD57$^+$ subset from CD27$^+$ precursors (**Fig. 1**). TCR-stimulation induced the differentiation of sorted CD8$^+$CD27$^+$CD57$^-$ TILs into a Perf^{hi}, CD27$^-$CD57$^-$ subset, but the cells differentiating from the CD27$^+$CD57$^+$ subset on average acquire higher perforin levels and killing function (**Fig. 1**). We also found that TGFβ1, produced by many melanomas, arrested the differentiation and cytotoxic activities of both the CD27$^+$CD57$^-$ and CD27$^+$CD57$^+$ subsets at the CD27$^+$ stage. Taken together, these findings may help explain why metastatic melanomas are often infiltrated with only early T_{EM} CD8$^+$ T cells that have limited ability to kill tumor cells. Thus, TGFβ1 may be a key suppressor of CTL differentiation in the tumor microenvironment (**Fig. 1**). However, other factors such as PGE_2, indoleamine-2,3-dioxygenase (IDO), IL-10, or inhibitory signaling through PD-1, may also play a role.[8]

Another interesting observation we made was that both the CD8$^+$CD27$^+$CD57$^+$ and CD8$^+$CD27$^+$CD57$^-$ T cells seemed to

ultimately differentiate into $CD27^-CD57^-$ (also $CD28^-$) cells subset with high perforin and killing activity. Are these the true end-stage or terminally-differentiated state of CTL that actually may be more NK-like, by expressing CD122, other NK receptors (NKG2D and high KIR levels)? These cells have been described before, but their origin is unknown.[9]

In conclusion, we characterized a novel subset of TILs in metastatic melanoma and breast cancer that seems to be locked in a transitional state between early T_{EM} and fully-differentiated CTL. Our study also underscores the need for a greater understanding of the state of CTL differentiation and activity in patients in relation to tumor control. Overall, there has been an over-emphasis on the role of cytokine release by $CD8^+$ T cells (e.g., IFNγ), which has generally not correlated with clinical efficacy during cancer immunotherapy. A case in point is the recent HIV vaccine trials where effective immunization correlated more with a gain of antigen-specific CTL phenotype and killing function rather than IFNγ production.[10]

References

1. Mortarini R, Piris A, Maurichi A, Molla A, Bersani I, Bono A, et al. Lack of terminally differentiated tumor-specific $CD8^+$ T cells at tumor site in spite of antitumor immunity to self-antigens in human metastatic melanoma. Cancer Res 2003; 63:2535-45; PMID:12750277.
2. Wu RC, Liu S, Chacon JA, Wu S, Li Y, Sukhumalchandra P, et al. Detection and Characterization of a Novel Subset of $CD8^+CD57^+$ T-cells in Metastatic Melanoma with an Incompletely-differentiated Phenotype. Clin Cancer Res 2012; 18(9):2465-2477; PMID:22307139; http://dx.doi.org/10.1158/1078-0432.CCR-11-2034.
3. Strioga M, Pasukoniene V, Characiejus D. $CD8^+$ $CD28^-$ and $CD8^+$ $CD57^+$ T cells and their role in health and disease. Immunology 2011; 134:17-32; PMID:21711350; http://dx.doi.org/10.1111/j.1365-2567.2011.03470.x.
4. Hoji A, Connolly NC, Buchanan WG, Rinaldo CR Jr. CD27 and CD57 expression reveals atypical differentiation of human immunodeficiency virus type 1-specific memory $CD8^+$ T cells. Clin Vaccine Immunol 2007; 14:74-80; PMID:17079436; http://dx.doi.org/10.1128/CVI.00250-06.
5. Brenchley JM, Karandikar NJ, Betts MR, Ambrozak DR, Hill BJ, Crotty LE, et al. Expression of CD57 defines replicative senescence and antigen-induced apoptotic death of $CD8^+$ T cells. Blood 2003; 101:2711-20; PMID:12433688; http://dx.doi.org/10.1182/blood-2002-07-2103.
6. Walker EB, Haley D, Petrausch U, Floyd K, Miller W, Sanjuan N, et al. Phenotype and functional characterization of long-term gp100-specific memory $CD8^+$ T cells in disease-free melanoma patients before and after boosting immunization. Clin Cancer Res 2008; 14:5270-83; PMID:18698047; http://dx.doi.org/10.1158/1078-0432.CCR-08-0022.
7. Anichini A, Molla A, Vegetti C, Bersani I, Zappasodi R, Arienti F, et al. Tumor-reactive $CD8^+$ early effector T cells identified at tumor site in primary and metastatic melanoma. Cancer Res 2010; 70:8378-87; PMID:20861189; http://dx.doi.org/10.1158/0008-5472.CAN-10-2028.
8. Polak ME, Borthwick NJ, Gabriel FG, Johnson P, Higgins B, Hurren J, et al. Mechanisms of local immunosuppression in cutaneous melanoma. Br J Cancer 2007; 96:1879-87; PMID:17565341; http://dx.doi.org/10.1038/sj.bjc.6603763.
9. Takayama E, Koike Y, Ohkawa T, Majima T, Fukasawa M, Shinomiya N, et al. Functional and Vbeta repertoire characterization of human $CD8^+$ T-cell subsets with natural killer cell markers, $CD56^+$ $CD57^-$ T cells, $CD56^+$ $CD57^+$ T cells and $CD56^-$ $CD57^+$ T cells. Immunology 2003; 108:211-9; PMID:12562330; http://dx.doi.org/10.1046/j.1365-2567.2003.01575.x.
10. Makedonas G, Betts MR. Living in a house of cards: re-evaluating $CD8^+$ T-cell immune correlates against HIV. Immunol Rev 2011; 239:109-24; PMID:21198668; http://dx.doi.org/10.1111/j.1600-065X.2010.00968.x.