Research Highlights

Highlights from the latest articles in T-cell-based immunotherapy

Rescuing tumor-specific T cells from cell death

Evaluation of: Norell H, Martins da Palma T, Leshner A *et al.*: Inhibition of superoxide generation upon T-cell receptor engagement rescues Mart-1(27–35)-reactive T cells from activation-induced cell death. *Cancer Res.* 69, 6282 (2009).

The development of effective T-cell-based immunotherapy to treat cancer patients requires the activation of a sufficient number of tumor antigen (TA)-specific T lymphocytes as well as their long-term persistence. Reactive oxygen species (ROS) play a critical role in regulating various T-cell processes, including survival, cytokine production and proliferation [1]. Norell and colleagues report that inhibiting ROS in TA-specific T cells, with the antioxidants Mn (III) tetrakis (5, 10, 15, 20-benzoic acid) and porphyrin (MnTBAP), reduced apoptosis induced by activation with antigen. ROS regulates T-cell death through various mechanisms such as damaging DNA and altering the expression of apoptosis-related proteins, which may in turn regulate the mitochondrial membrane potential [2].

Accordingly, Norell and colleagues demonstrate that inhibiting ROS in TA-activated CD8⁺ T cells reduced the disruption of mitochondrial membrane integrity and decreased DNA fragmentation, as measured by the level of deoxycytidine in ssDNA. These results are significant for two reasons. First, inhibiting ROS may improve the expansion of TA-specific T cells for adoptive T-cell transfer therapy. Second, rescuing T cells from ROS-mediated apoptosis *in vivo* may have a direct impact on memory T-cell development, which may have a key role in the long-term surveillance of

tumor cells within the host. While the physiological significance of inhibiting ROS radicals on memory T-cell development was not explored in these studies, Vig *et al.* demonstrate that T cells lacking the inducible nitric oxide synthase promoted the generation and persistence of T-cell memory [3]. This suggests that regulating free radicals in T cells may be a useful strategy for manipulating memory T-cell development. Importantly, ROS inhibition did not impair IFN- γ production or the expression of CD107, a surrogate marker for degranulation. Although the exact signaling mechanisms by which ROS reduces apoptosis have yet to be fully characterized, Norell implicates the signaling intermediary, c-Jun NH₂-terminal kinase (JNK), in this process. Interestingly, inhibiting JNK reduced T-cell apoptosis but, in contrast to inhibiting ROS, it also impaired cytokine production. For future studies it would be beneficial to understand the effects of delivering ROS inhibitors directly to the TA-specific T cell *in vivo*.

In addition, T cells have a short survival span in response to various stresses such as Fas-mediated activation-induced cell death, growth factor withdrawal-induced death and the disruption of mitochondrial membrane potential. In conclusion, the importance of T-cell persistence over expanded periods of time is important, playing a key role in the prevention of tumor recurrence and holding potentially lethal cancer cells in check over time.

The development of cellular mechanisms that have the capacity to inhibit T-cell death may possibly enhance our ability to effectively treat cancer patients at the immunologic level, ultimately leading to an improved clinical outcome for patients [1,4].

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Enhancing melanoma immunotherapy using T-cell clones

Evaluation of: Khammari A, Labarrière N, Vignard V *et al.*: Treatment of metastatic melanoma with autologous Melan-A/MART-1-specific cytotoxic T-lymphocyte clones. *J. Invest. Dermatol.* DOI:10.1038/jid.2009.144 (2009) (Epub ahead of print).

“...we are beginning to find ways of concomitantly increasing the overall response rates for patients with metastatic disease in a cost-effective fashion...”

An effective treatment strategy for patients with metastatic melanoma has been exceedingly difficult to find. The history of immunotherapy for melanoma has been interesting, with sequential advances in both our understanding and armamentarium of treatment options. What began as stimulating the host immune system with high-dose IL-2 therapy has now developed into complex, but more effective, regimens of immune activation. The report by Khammari *et al.* highlights the efficacy of an autologous, tumor antigen (MART-1)-specific immunotherapy with cytotoxic T-lymphocyte clones [1]. Although the trial is very small, with only 14 patients treated, they report a high objective response rate of 50% (43% of partial and complete response, 7% with stable disease), with two patients achieving long-term remissions. This is within the range of similarly astounding results when compared with Dudley *et al.*, who report a range of overall objective response rates of 45–72% [2].

Although the conceptual approach to treatment is similar, this small Phase II trial differs from the latter trials in that

they did not pre-treat patients with a non-myeloablative regimen prior to autologous T-cell infusion. Second, this group selected the T-cell clones to be infused based upon their ability to secrete IL-2 in response to HLA-matched melanoma cells expressing the target antigen of interest. The former may account for the differences noted in immunologic surveillance of Melan-A-specific T cells. Nonetheless, we are beginning to find ways of concomitantly increasing the overall response rates for patients with metastatic disease in a cost-effective fashion, as the regimens currently utilized to achieve such high clinical response rates can be cost prohibitive, limited to facilities with the resources to support a dedicated team for such efforts, such as the National Cancer Institute. In conclusion, this article highlights recent advances in our current options for patients with metastatic melanoma, with the authors reporting a very promising objective overall response rate that will need to be reproduced in a larger study population.

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Phosphotumor antigen-specific CD4 T cells

Evaluation of: Depontieu FR, Qian J, Zarling AL *et al.* Identification of tumor-associated, MHC class II-restricted phosphopeptides as targets for immunotherapy. *Proc. Natl Acad. Sci. USA* 106(29), 12073–12078 (2009).

Our understanding of how the immune system recognizes malignant cells has led to the development of novel approaches to treat cancer patients. In further pursuit of identifying more favorable and discriminating MHC class II-restricted tumor targets, Depontieu and colleagues have identified a novel group of tumor-derived epitopes expressed on human cancer cells [1]. They characterized 175 HLA-DR-associated phosphopeptides derived from melanoma and Epstein–Barr virus (EBV)-transformed B cells, most of which contained a single phosphorylated residue. The majority of phosphopeptides appeared to be derived from proteins known to be critical for various biological processes, including metabolism, cell division and signal transduction, suggesting perhaps a higher level of expression for these proteins (and phosphopeptides) in cells experiencing rapid growth. To investigate more closely the relevance for activating tumor-specific T cells for enhancing immunotherapeutic approaches against melanoma, the authors generated HLA-DR-restricted CD4 T cells specific for a phosphorylated melanoma-associated antigen recognized by T cells (p-MART-1) peptide. CD4 T cells showed reactivity for p-MART-1-pulsed antigen-presenting cells as well as for patient-derived melanoma cells, but failed to recognize target cells expressing wild-type (nonphosphorylated) MART-1. Specificity for HLA-DR by CD4 T cells was further confirmed using blocking anti-MHC monoclonal antibodies.

Applying such knowledge to efficiently activate tumor-specific T cells against these and other post-translationally [2] modified epitopes is not without its challenges. First, the activation of sufficient numbers of tumor-specific T cells with efficient cytotoxic activity intended for adoptive T-cell therapy has proven to be cumbersome and costly. Although the identification of modified tumor epitopes has renewed interest in protein-based vaccine strategies, the technical limitations for producing recombinant proteins with the proper modifications will be a major drawback. Similarly, even though peptide-based vaccines have shown promise in several clinical studies, the results have been less than impressive, with response rates below 5% in most cases [3]. Finally, DNA-based approaches intended to activate antigen-specific T cells will not be able to target phosphopeptide-specific T cells. Despite these challenges, the identification of MHC class II-restricted phosphopeptides adds to the array of T cells that can presumably become specifically activated to destroy cancer cells. In addition, given the high degree of dysregulated signal transduction pathways in cancerous cells, the generation of phosphotumor epitopes may serve to better target T-cell responses against tumor cells.

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PD-1 and impaired antitumor T cell responses in melanoma

Evaluation of: Ahmadzadeh M, Johnson LA, Heemskerk B *et al.*: PD-1 and impaired antitumor T cell responses in melanoma tumor antigen-specific CD8 T cells infiltrating the tumor express high levels of PD-1 and are functionally impaired. *Blood* 114(8), 1537–1544 (2009).

“...the tumor microenvironment enhances the expression of PD-1 on tumor-infiltrating lymphocytes, and impairs the antitumor immune responses.”

The use of tumor antigen (TA)-specific T cells for adoptive T-cell therapy to treat cancer patients has not demonstrated the success many had hoped for [1]. Despite the fact that transferred TA-specific T cells are found in circulation as well as in melanoma lesions, tumors continue to grow in some patients [2]. To help clarify T-cell dysfunction within tumors, Ahmadzadeh and colleagues examined programmed death receptor (PD)-1 expression on tumor-infiltrating lymphocytes (TILs) in metastatic melanoma lesions [3]. The engagement of PD-1 (CD279) on T cells, including TA-specific cells, reduces their capacity to proliferate and produce effector cytokines during the early stages of T-cell activation [4–6]. Ahmadzadeh *et al.* found that the expression levels of PD-1 on CD4 and CD8 TIL as well as the overall frequency of T cells expressing PD-1 were significantly higher as compared with T cells derived from normal tissues (liver, lung and spleen) and peripheral blood in the same patients or healthy donors. PD-1⁺ TILs were impaired in their ability to produce effector cytokines, such as IFN- γ and IL-2, as compared with PD-1⁺ TILs and PD-1⁺ peripheral blood T lymphocytes derived from the same patient. These findings suggest that the tumor microenvironment enhances the expression of PD-1 on TILs, and impairs the antitumor immune responses. However, it is worth noting that TILs had a population of both PD-1⁺ and PD-1⁺ cells, for which the question arises as to whether the

tumor microenvironment induces PD-1 expression or whether PD-1⁺ T cells preferentially accumulate within the tumor itself. Unfortunately, the factors that can induce PD-1 expression on T cells remain elusive. The authors also report that PD-1⁺ TIL failed to express CD127 (IL-7 receptor α -chain) but expressed HLA-DR⁺ and cytotoxic T lymphocyte-associated antigen 4 (CTLA-4) – another negative regulator. Thus, whether PD-1 stimulation is directly responsible for T cell dysfunction or simply serves as a phenotypic marker have yet to be elucidated. Collectively, these studies highlight one potential mechanism by which TA-specific T cells may lose their ability to destroy cancerous cells and could allow for novel therapeutic approaches to treat cancer patients via blockage of these negative signals.

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