were present only when tumors were undergoing neovascular growth, and immunohisotchemistry confirmed that iron-containing cells within the tumor and its periphery were positive for 2 endothelial cell markers, CD-31 and von Willebrand factor. Although the study did not control for the potential leakage of iron from the transplanted cells and subsequent redistribution to vasculature, there are significant data from other studies to strongly suggest that this is not the mechanism in place.[3] Furthermore, the use of labeled nonviable cells for control animals supports the authors' conclusions that ex vivo–labeled progenitor cells migrate and differentiate into endothelial cells and are responsible for the signal changes observed.

Others have certainly demonstrated the use of various imaging modalities including MRI to track the migration of cells in vivo.[4] However, the results of Anderson et al are very exciting because they are the first to demonstrate the utility of the approach for tracking the migration and differentiation of endothelial progenitor cells into tumor vasculature. In contrast to other noninvasive techniques used to assess microvessel density (e.g., perfusion imaging), this technology allows not only assessment of neovasculature but also the ability to noninvasively visualize functioning, genetically altered cells with resolution not obtainable by any other modality.

As science moves toward more novel approaches for delivering genes to target tissues, clinical trials will require a means to assess dosage and frequency of cell transplants in order to optimize therapy and predict outcome and side effects. Approaches such as those described by Anderson et al will be important in helping to assess these parameters in vivo. ■

REFERENCES

1. Folkman J. Angiogenesis: therapeutic implication. N Engl J Med. 1971;285:1182-1186.
2. Asahara T, Masuda T, Takahashi T, et al. Bone marrow origin of endothelial progenitor cells responsible for postnatal vasculogenesis in physiological and pathological neovascularization. Circ Res. 1999;85:221-228.
3. Kircher MF, Mahmood U, King RS, et al. A multimodal nanoparticle for preoperative magnetic resonance imaging and intraoperative optical brain tumor delineation. Cancer Res. 2003;63:8122-8125.
4. Lewin M, Carlesso N, Tung CH, et al. Tat peptide-derivatized magnetic nanoparticles allow in vivo tracking and recovery of progenitor cells. Nat Biotechnol. 2000;18:410-414.

IMMUNOBIOLOGY

Comment on Powell et al, page 241

Long live T cells

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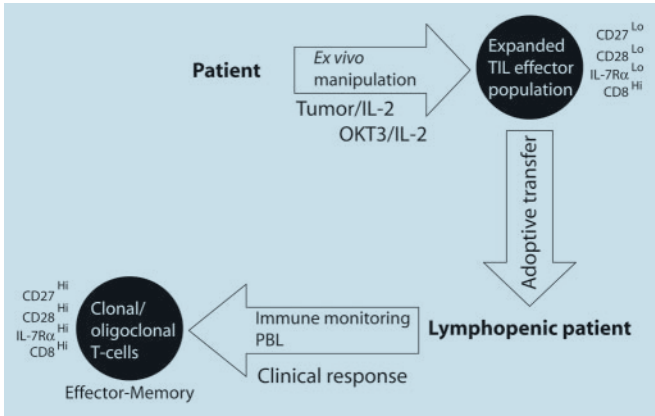
The clinical responses achieved by adoptive immunotherapy correlate with the in vivo persistence of infused melanoma-specific T cells that have up-regulated expression of IL-7Rα and have recapitulated an effector memory cell phenotype.

The premise and promise of T-cell therapy stems from the ability to selectively augment a recipient's immune response against a desired pathologic antigen. Adoptive immunotherapy for melanoma is an attractive approach, for it is well accepted that T cells can recognize melanoma-associated antigens. The translation of this concept into T-cell trials with therapeutic value for patients with metastatic tumor has recently been accomplished by Rosenberg and colleagues[1] using heterogeneous populations of melanoma-specific T cells, ex vivo expansion and infusion of large numbers of T cells, co-infusion of $CD4^+$ and $CD8^+$ T cells, a pre-immunotherapy lymphodepletion regimen, and repeated administration of exogenous interleukin 2 (IL-2).[1-3] In this issue of *Blood*, the authors follow the fate of adoptively transferred T cells in 6 responding melanoma patients and develop a rationale for their observed clinical responses.

To generate the numbers of tumor-specific T cells for adoptive immunotherapy trials, Powell and colleagues have developed a protocol to grow tumor-infiltrating lymphocytes (TILs) in vitro by stimulating with autologous resected tumor and high-dose IL-2, followed by rapid numerical expansion mediated by OKT3 (anti-CD3) and IL-2 that delivers a potent mitogenic signal. A potential drawback to this culturing methodology is that the expanded $CD8^+$ T cells typically down-regulate cell-surface expression of CD27, CD28, IL-7Rα (CD127), as well as CD62L and CCR7. This phenotype is consistent with differentiated effector T cells, which are associated with a decreased ability to proliferate and a reduced potential to survive in vivo. However, it appears from their in vivo data that not all of the cultured T cells were terminally differentiated.

Adoptive transfer of these melanoma-specific T cells (identified by tetramer-binding and/or Vβ T-cell receptor expression) in some lymphopenic recipients resulted in rapid in vivo expansion followed by sustained homeostatic proliferation. Serial sampling from peripheral blood of patients demonstrated that the infused T cells had increased expression of the costimulatory molecules CD27, CD28, and IL-7Rα and this was associated with long-term persistence of effector memory T cells (figure). Extrapolating from these data, it appears that the infused T cells that survive are capable of responding to IL-7, a cytokine that is associated with transition of effector $CD8^+$ T cells to memory cells and regulating the size of the T-cell pool by helping to compensate for T-cell depletion.[4,5] However, other facets of the National Cancer Institute (NCI) T-cell trial may contribute to survival of the infused $CD8^+$ T cells, such as co-infusion of $CD4^+$ T-helper cells, as well as removal of regulatory T-cell subsets in the lymphopenic recipient. It remains to be seen if the effector memory T



Schematic of the adoptive transfer of a population of in vitro–expanded melanoma-specific $CD8^+$ T cells in the lymphopenic recipient and, following contraction of the infused effector cells, emergence of clonal/oligoclonal effector memory T cells.

cells will continue to persist and whether these lymphocytes can function to control/prevent melanoma growth over the long term. Indeed, to help correlate the surrogate data obtained by immune monitoring with clinical outcome, these studies will need to evolve from sampling of peripheral blood to evaluating the immunobiology of adoptively transferred T cells residing in the tumor microenvironment, preferably using tools that also probe the functional activity of sampled T cells.

In this issue of *Blood*, Powell and colleagues highlight the power of the in vivo processes to select for a subpopulation of transferred T cells, such as memory-cell precursors, that can selectively survive in a lymphopenic host, and have important implications for the field of adoptive immunotherapy in general. Many adoptive immunotherapy trials numerically expand T cells ex vivo by providing a stimulatory signal through endogenous αβ T-cell receptor or by cross-linking CD3, in the presence of IL-2. The data in this paper will help reassure investigators that these in vitro growth conditions may not be an impediment to generating long-lived T cells in vivo. However, it is still to be determined whether these results from patients with melanoma can be translated to other T-cell therapy trials employing lymphodepleting-conditioning regimens, including clinical trials infusing T cells with specificity directed through chimeric immunoreceptors. Hopefully, such experiments will continue to delineate critical variables contributing to the in vivo survival of transferred T cells in the lymphopenic and lymphoreplete environments. Furthermore, additional studies will be needed to test whether adoptive immunotherapy can be improved by the a priori identification, or enforced expression, of receptors (such as IL-7Rα$^+$) that endow T cells with improved in vivo survival. As these future adoptive immunotherapy trials are implemented, investigators and regulators will need to balance the relative risks and benefits obtained from transferring homogeneous well-defined clonal populations of antigen-specific T cells versus infusing heterogeneous bulk populations potentially containing antigen-reactive T cells capable of taking advantage of the recipient's lymphopenic milieu. ■

REFERENCES

1. Rosenberg SA, Dudley ME. Cancer regression in patients with metastatic melanoma after the transfer of autologous antitumor lymphocytes. Proc Natl Acad Sci U S A. 2004;101(suppl 2):14639-14645.

2. Dudley ME, Wunderlich JR, Shelton TE, Even J, Rosenberg SA. Generation of tumor-infiltrating lymphocyte cultures for use in adoptive transfer therapy for melanoma patients. J Immunother. 2003;26:332-342.

3. Dudley ME, Wunderlich JR, Robbins PF, et al. Cancer regression and autoimmunity in patients after clonal repopulation with antitumor lymphocytes. Science. 2002; 298:850-854.

4. Kaech SM, Tan JT, Wherry EJ, Konieczny BT, Surh CD, Ahmed R. Selective expression of the interleukin 7 receptor identifies effector CD8 T cells that give rise to long-lived memory cells. Nat Immunol. 2003;4:1191-1198.

5. Jameson SC. Maintaining the norm: T-cell homeostasis. Nat Rev Immunol. 2002;2:547-556.

HEMATOPOIESIS

Comment on Masuya et al, page 95

A Fli in the ointment

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Transcription factors that are aberrantly activated in leukemias often play a seminal role in blood lineages other than the malignant cell population. Knowing the full spectrum of transcription factor actions should inform our understanding of leukemia pathogenesis.

Leukemogenic chromosomal translocations commonly perturb transcription factor (TF) genes that are normally expressed in a restricted number of blood cell lineages. Many studies in the 1990s demonstrated the physiologic roles of these TFs in defining the character of their host cell types.[1] Subsequent investigation revealed additional roles in other lineages and surprising interactions among factors with ostensibly distinct functions.[2] Indeed, TFs historically associated with a single or few closely related cell types may also be expressed, albeit at different levels or to different ends, in other lineages and especially in pluripotent progenitors. It is still unclear which class of TF activities is responsible for the leukemias that result from their misexpression.

In this issue of *Blood*, Masuya and colleagues add a new twist to an old story on experimental erythroleukemia in mice. Most malignant clones induced by integration of the Friend leukemia virus activate expression of Friend leukemia integration 1 (Fli-1), an Ets-family TF that may regulate transcription of many genes in the sibling erythroid and megakaryocytic lineages.[3] Mice lacking *Fli-1* die before definitive hematopoiesis occurs in development, though it is possible to detect defects in megakaryocyte and erythoid maturation. Nuanced changes in other blood cells, by contrast, were less evident and it is this aspect that Masuya and colleagues address in chimeric mice they created using both embryo aggregation and conventional bone marrow transplantation. In their experiments, cells derived from *Fli-1*$^{-/-}$ or *Fli-1*$^{+/-}$ embryos made a measurably smaller contribution toward circulating neutrophils and monocytes and possibly generated more natural killer (NK) cells than expected. The results suggest unanticipated functions for Fli-1 in aspects of hematopoiesis besides megakaryocyte differentiation; the experimental design presumes that defects arising in the absence of Fli-1 are intrinsic to the affected lineage.

Studies of this kind constitute an essential step toward full understanding of TF functions. The conclusions of the Masuya et al study, however, rest on analysis of very few animals and the effect of Fli-1 deficiency on leukocytes seems subtle and confined to the peripheral blood population: cell proportions and morphology in the bone marrow are unaffected. Moreover, the authors postulate a dependence on *Fli-1* gene dosage that is not revealed independently in germline heterozygote mice. Each of these caveats will need to be addressed in future studies. Meanwhile, the results invite immediate consideration of the implications for erythroleukemia pathogenesis.

Different Friend virus strains induce similar hematologic changes and integrate near remarkably few gene loci. Besides Fli-1, another Ets-family TF, Spi-1 or Pu.1, is commonly involved, as is the p45 subunit of nuclear factor–erythroid 2 (NF-E2). Can