The combination of the induction of lymphopenia and vaccination and/or T cell transfer is garnering much attention for cancer treatment. Preclinical studies have shown that the induction of lymphopenia by chemotherapy or radiation can enhance the antitumor efficacy of several distinct, cell-based immunotherapeutic approaches. The mechanism(s) by which such enhancement is achieved are being intensively studied, yet there is much opportunity for improvement. The animal studies reported by Wrzesinski and colleagues in this issue of the JCI are a promising and timely step in this direction (see the related article beginning on page 492). The authors have evaluated both the effect of increasing the intensity of lymphodepletion and the influence of HSC transfer on the in vivo function of adoptively transferred CD8+ T cells. We discuss their results in light of the evolving field and their implications for advancing cell-based immunotherapies for cancer.

Current immunization approaches attempt to activate and expand the tumor-reactive T cell population in hosts with an intact immune system. There is much evidence that within the immune system of cancer patients, tumor-induced suppression and immune-based regulatory factors are present that may limit the effectiveness of vaccine-induced, tumor-specific T cells (1). An alternative approach is to induce lymphopenia (a reduction in lymphocyte number) in hosts, allowing residual host or transferred naive or antigen-specific donor T cells to undergo homeostasis-driven proliferation to restore the memory T cell compartment. Several potential advantages are offered by this strategy. For example, in addition to eliminating inhibitory immune cells in the host such as Tregs, lymphomyeloid reconstitution may overcome inherent defects in T cell signaling and may strengthen the costimulatory functions of APCs (2). Induction of lymphopenia can lead to an increased production and availability of immune response-stimulating cytokines such as IL-7 and IL-15, resulting in enhanced CD8+ T cell activity (3, 4). Other studies have shown enhanced T cell trafficking into tumors after induction of lymphopenia (5, 6), as well as enhanced intratumoral proliferation of effector cells (7). It is postulated that vaccination during homeostasis-driven proliferation may serve to educate the developing T cell repertoire and lead to enhanced T cell memory against tumor-associated self antigens (8, 9).

Common methods to induce lymphopenia include treatment with low-dose total body irradiation (TBI) that produces mild, reversible myelosuppression (hence nonmyeloablative) or treatment with chemotherapeutic drugs such as cyclophosphamide (Cy) alone or in combination with fludara-
T cells and injection of a melanoma vaccine in RAG1-deficient mice. DC-based vaccines are particularly attractive as a treatment adjunct, as homeostasis-driven proliferation has been shown to be dependent on interactions of T cells with self-peptide MHC on DCs (19), and such vaccines can boost the antitumor activity of adaptively transferred, tumor antigen peptide–specific T cells in vivo (20). Lymphodepleting chemotherapy in combination with adoptive transfer of naive T cells and DC-based immunotherapy can lead to rejection of established melanoma (14). The most effective antitumor immunity is induced when vaccination and reconstitution are performed concomitantly, as delayed vaccination may result in T cells with less antitumor potency (14).

In the mouse, lymphopenia drives not only the proliferation of CD44hiCD62Llo (CD44hiCD62Llo) effector memory T (TEm) cells, but also induces naive T cells undergoing homeostasis-driven proliferation to convert to a memory or activated state (21–23). Both TEm cells and “central” memory T (Tcm) cells, expressing high levels of CD62L and C-C motif chemokine receptor 7 (CCR7), have been shown to be important for long-lasting immune responses (24, 25). While T cells are phenotypically Tcm cells during homeostatic proliferation, DC-based vaccines can accelerate the development of TEm cells (26). TEm cells proliferate better in response to antigenic stimulation, leading to enhanced protection against antigenic challenge (27). A recent study demonstrated in melanoma-bearing mice that cytokine therapy in combination with adoptive T cell transfer and a DC-based vaccine in the setting of lymphopenia can lead to a higher number of TEm cells, which correlated with long-term survival (28).

In cancer patients, nonmyeloablative induction of lymphopenia enhanced the efficacy of adoptively transferred, tumor antigen–specific T cells (29). Transfer of melanoma-associated antigen recognized by T cells 1–specific (MART-1–specific), autologous tumor-infiltrating lymphocytes (TILs) and high-dose IL-2 therapy after chemotherapy-induced lymphopenia resulted in the rapid expansion in vivo of a clonal population of T cells specific for the MART-1 antigen and resulted in the destruction of metastatic tumors and induction of autoimmunity against nor-

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**Figure 1**
Schematic of the promotion of expansion and function of adoptively transferred antitumor CD8+ T cells following myeloablation and HSC rescue. HSC transplant, given as part of the myeloablative regimen, can significantly augment the expansion and the antitumor impact of adoptively transferred self/tumor antigen–reactive T cells. Myeloablation effectively removes host inhibitory cells, opens up available space (the so-called Lebensraum effect), and destroys cells serving as a cytokine sink. The operative mechanism by which HSCs positively impact the transferred T cells is currently unknown but could include the production of APCs and T cell homeostatic cytokines (e.g., IL-7 and IL-12), GR1, suppressive monocytes; HSC, lin-c-kit+ HSC; pmel-1 TCR, gp100 melanoma–associated antigen-specific T cell; Treg, CD4+CD25+Foxp3+ Tregs; tumor, melanoma expressing gp100.
In other clinical studies, strategies have included transfer of HSCs from a donor immunized with tumor antigen (idiotype proteins) to an allogeneic HSC recipient to avoid relapse in the setting of multiple myeloma (43, 44). Moreover, combination immunotherapy consisting of a single early posttransplantation infusion of in vivo vaccine-primed and ex vivo costimulated autologous T cells followed by post-transplantation booster immunizations can improve the severe immunodeficiency associated with high-dose chemotherapy and can lead to the induction of clinically relevant immunity in adults within a month after transplantation (45). In cases where the functional quality of T cells for adoptive transfer against tumors or microbial/viral infections is compromised, rescue can be achieved by the use of IL-15 (46), anti-CD40 (47), or program death 1 (PD-1) engagement blockade (48), potentially allowing for a broader use of adoptive immunotherapy in these settings.

HSCs appear to be the key

The study by Wrzesinski et al. reported in this issue of the *JCI* shows that prior treatment with a myeloablative TBI dose requiring an HSC transplant can enhance both the expansion of the adoptively transferred, tumor-reactive T cell population and the efficacy of tumor treatment in mice as compared with a nonmyeloablative TBI dose not requiring an HSC transplant (13). Importantly, the mere reduction of the levels of regulatory host elements and the presence in small numbers after TBI-mediated regulatory cells that dampen the immune response (27). Transfer of purified T cells from the bone marrow of a naive donor may be more reactive to vaccination with self antigens than the endogenous T cells remaining after nonmyeloablative chemotherapy in a tumor-bearing host. Indeed, homeostasis-driven proliferation restores only the memory T cell compartment, whereas thymopoiesis is required to reconstitute the naive T cell compartment (36). After whole BMT, both homeostatic proliferation of resident T cells in the bone marrow and thymopoiesis together lead to reconstitution of the T cell compartment. In addition, bone marrow contains a high percentage of T<sub>cm</sub> cells (37). Whereas induction of T<sub>cm</sub> cells is important for a peripheral immune response, T<sub>cm</sub> cells are superior in trafficking to peripheral lymph nodes and inducing strong systemic immunity (27). Transfer of purified T cells from the bone marrow of tumor lysate–pulsed DC–vaccinated mice could lead to regression of breast cancer and melanoma (32). In another study, adoptive transfer of T<sub>cm</sub> cells was more effective than transfer of T<sub>cm</sub> cells at inducing tumor regression in a murine model of melanoma (24), indicating the importance of CD8<sup>+</sup> T<sub>cm</sub> cells for the induction of strong, systemic antitumor immunity.

In the setting of lymphopenia after HSC transplantation, adoptive T cell immunotherapy as a prophylactic or strategy for treating CMV, EBV, and adenovirus infections after transplantation has been conducted with promising outcomes (38–40). Strategies for employing T cells specific for minor histocompatibility antigens (mHA) to augment the graft-versus-leukemia effect that contributes to tumor eradication are also being employed (41, 42).
independent of MHC class I and independent of host T cells. Although in some models, expansion of transferred, experienced T cells is APC independent and IL-7 and IL-15 dependent, Zaft et al. (56) have shown that it can be dependent on resident CD11c+ DCs. Thus, HSC-derived CD11c+ DCs or other APCs might affect homeostasis of tumor-specific effector T cells. Whether costimulation is provided through soluble factors, as Wrzesinski and colleagues speculate (13), or cell surface molecules such as CD40, CD80, CD86, or CD134 ligand also remains to be elucidated. With respect to regulatory cells, HSC-derived APCs provide survival signals to residual radioreistant regulatory cells, including Tregs and NKT cells.

Third, the authors’ supplemental data suggest that non ablative irradiation plus vaccination appears to be as effective as ablative irradiation and HSC transplantation: what then is the benefit of employing the added benefit of myeloablation (including in the setting of nonmyeloablative conditioning) (31).

Fourth, how generalizable are the findings? Although the transgenic mouse model in which the pmel-1 TCR is expressed—with the very high frequency (~90%) of murine gp100 melanoma-associated antigen-reactive CD8+ T cells—is attractive from the standpoint of assay sensitivity/specificity as well as its ability to tease out the operative mechanisms involved, it is unknown whether the added benefit of myeloablation and HSC transplant can be replicated in a more standard model, e.g., in conventional C57BL/6 mice undergoing lethal TBI and syngeneic HSC transplantation followed by the adoptive transfer of TILs containing a polyclonal population of tumor-specific CD4+ and CD8+ T cells. The latter would be more akin to the ongoing clinical trial in melanoma patients (30). Also of importance, earlier clinical studies involving the adoptive transfer of cloned CD8+ T cells did not result in any objective responses or any persistence of the transferred cells (including in the setting of nonmyeloablative conditioning) (31).

While it will be important to define the exact mechanism whereby HSCs can promote the expansion of adoptively transplanted antitumor CD8+ T cells, perhaps more important will be to put these exciting findings to the test in the clinic. Given the initial promising clinical results to date of the adoptive transfer of TILs (30) and TCR gene-modified peripheral blood lymphocytes in nonmyeloablative, lymphopenic melanoma patients (58), the stage is now set to carry the strategy further in the setting of myeloablation with HSC transplant rescue.

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Nutrient overload induces obesity, a primary risk factor for type 2 diabetes. Ribosomal biogenesis and protein synthesis, which are controlled by the mammalian target of rapamycin (mTOR), are primary energy-consuming processes in cells. mTOR phosphorylates and inactivates members of the eukaryotic translation initiation factor 4E–binding (eIF4E-binding) protein (4E-BP) family, which are translational repressors of 5′ cap–dependent protein synthesis. In this issue of the JCI, Le Bacquer et al. report that simultaneous deletion of both 4E-BP1 and 4E-BP2 in mice results in insulin resistance, decreased energy expenditure, and increased adipogenesis (see the related article beginning on page 387). These findings link protein synthesis, insulin sensitivity, and body weight.

Food (energy) shortage is a constant threat to the survival of a species. Individuals who can efficiently maintain their body weight via energy conservation have an increased chance of survival and propagation during times when food supply is limited. Nutrients and hormones activate multiple evolutionarily conserved signaling pathways that govern the balance between energy intake and expenditure. Mammalian target of rapamycin (mTOR) is a well-conserved serine/threonine protein kinase that functions as an intracellular nutrient sensor to control protein synthesis, cell growth, and metabolism. In this issue of the JCI, Le Bacquer et al. demonstrate that the eukaryotic translation initiation factor 4E–binding (eIF4E-binding) protein (4E-BP) family of translational repressors, which are physiologic substrates of mTOR, play a key role in regulating body weight and glucose homeostasis in mice (1).

**mTOR regulation of energy and glucose metabolism**

mTOR is a member of the phosphoinositide kinase–related kinase family and is activated by nutrients (e.g., branched-chain amino acids) as well as by metabolic hormones, growth factors, and cytokines. mTOR binds to other regulatory components to form 2 distinct multiprotein complexes. The first complex, mTORC1, contains mTOR, regulator–associated protein of mTOR (Raptor), and G protein β subunit–like protein (GβL). The second complex, mTORC2, contains mTOR, rapamycin-insensitive companion of mTOR (Rictor), mammalian stress–activated protein kinase–interacting protein 1 (mSin1), and GβL (Figure 1). The adaptor proteins Raptor and Rictor determine the substrate specificity of mTORC1 and mTORC2, respectively. mTORC1 specifically phosphorylates ribosomal protein

**A link between protein translation and body weight**

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**Nonstandard abbreviations used:** DKO, double knockout; 4E-BP, eIF4E-binding protein; eIF, eukaryotic translation initiation factor; IRS, insulin receptor substrate; mTOR, mammalian target of rapamycin; mTORC, mTOR complex; PDK1, phosphoinositide-dependent protein kinase 1; S6K, ribosomal protein S6 kinase; S6K, untranslatable region.

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