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Toward improved immunocompetence of adoptively transferred CD8$^+$ T cells

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Adoptive transfer of autologous or allogenic T cells to patients is being used with increased frequency as a therapy for infectious diseases and cancer. However, many questions remain with regard to defining optimized procedures for preparation and selection of T cell populations for transfer. In a new study in this issue of the *JCI*, Gattinoni and colleagues used a TCR transgenic mouse model to examine in vitro–generated tumor antigen–specific CD8$^+$ T cells at various stages of differentiation for their efficacy in adoptive immunotherapy against transplantable melanoma (see the related article beginning on page 1616). The results confirm that CD8$^+$ T cells progressively lose immunocompetence with prolonged in vitro cultivation and suggest that effector CD8$^+$ T cells alone may be considerably less potent at protecting hosts with advanced tumors than are less differentiated T cells.

In addition to the well-established donor lymphocyte infusion (DLI) approach to treating leukemia relapse after HSC transplantation, adoptive cell transfer therapy (ACT) is also being developed to treat EBV- and CMV-associated diseases, and more recent initiatives have focused on the use of ACT to treat solid human cancers, primarily melanoma (1–5). Unfortunately, in vitro–cultured antigen-specific T cells, particularly T cell clones, often die only a few hours after adaptive transfer and generally do not survive more than a matter of days, which limits treatment efficacy (6, 7). By contrast, T cells adoptively transferred directly from donor to recipient show increased survival rates and are more likely to become immunoprotective; this has been confirmed by transfer experiments using T cells from TCR transgenic mice, which provide unprecedented amounts of naive antigen-specific donor T cells and thus circumvent the need for in vitro T cell cultivation (8). In humans, the most durable form of ACT is DLI, which usually involves direct peripheral wbc transfer from the allogenic donor to the leukemia patient who has previously received HSCs from the same donor. Most patients with solid tumors, however, have never undergone allogenic stem cell transplantation and thus cannot receive donor cells, but instead depend on transfer of autologous cells. These cells must be selected and/or enriched in cell cultures in order to obtain large numbers of T cells with appropriate antigen specificity. One of the great challenges in ACT lies in the development of optimal procedures for lymphocyte selection and preparation.

Nonstandard abbreviations used: ACT, adoptive cell transfer therapy; CD62L, CD62 ligand; DLI, donor lymphocyte infusion; TREC, TCR rearrangement excision circle.

Conflict of interest: The authors have declared that no conflict of interest exists.

Citation for this article: *J. Clin. Invest.* **115**:1467–1469 (2005). doi:10.1172/JCI25427.

T cell differentiation

The study by Gattinoni et al. (9) in this issue of the *JCI* addresses the question of whether progressive CD8$^+$ T cell differentiation toward an effector T cell phenotype is associated with changes in the cells' capacity to protect the host from disease. The study provides detailed insight into the relationship among the duration of in vitro T cell culture, the functional and phenotypic characteristics of T cells at various stages of differentiation, and their immunocompetence upon adoptive transfer. The authors performed sequential rounds of in vitro stimulation in order to promote progressive CD8$^+$ T cell differentiation. The longer the T cells were stimulated and cultured in vitro, the more they acquired the

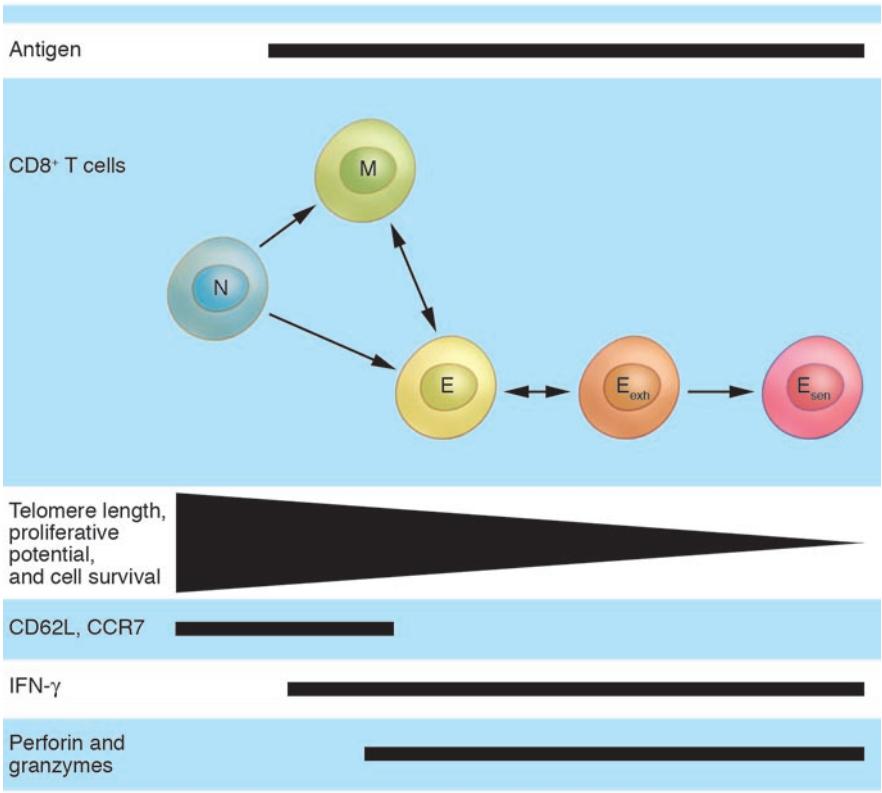

Figure 1
In vivo activation of CD8+ T cells is associated with downregulation of lymph node homing receptors, acquisition of effector functions, and migration to diseased tissues. When naive (N) T cells encounter antigen, they become activated and differentiate to effector (E) and memory (M) T cells. With increased antigen stimulation and T cell activation and differentiation, effector T cells progressively lose telomere length and proliferative potential, may become exhausted (E_{exh}) and/or senescent (E_{sen}), and/or may undergo apoptosis. Lymph node homing receptor CD62L and CC chemokine receptor 7 (CCR7) are downregulated when T cells differentiate to effector T cells. At the same time, effector T cells acquire the expression of IFN-γ and the cytolytic proteins perforin and granzymes. The figure represents a simplified scheme; for more comprehensive overviews see, for example, refs. 14–16.

properties of effector T cells (e.g., expression of IFN-γ, perforin, and granzymes), but the less they were capable of controlling large, established tumors in recipient mice. It seems paradoxical that despite their enhanced effector properties, effector CD8+ T cells appeared to be more than 100 times less effective for in vivo treatment than short-term cultures containing early intermediates of T cell differentiation. At first sight, the results of these ACT experiments appear to contradict previous observations that highly differentiated effector CD8+ T cells exert strong effector functions and are associated with lymphocyte-mediated protection from disease (10). Because of the relatively poor ability of the highly differentiated effector CD8+ T cells to provide tumor protection upon transfer to recipient mice, Gattinoni et al. propose that in vitro expression of IFN-γ, perforin, and granzymes, in addition to low proliferative capacity, are characteristic properties of "impaired" cells (9). If the cells were indeed impaired in their ability to destroy tumor cells, was it because they were generated in vitro? Although in vitro–cultured transgenic T cells acquire functional attributes and phenotypic cell-surface markers similar to those of effector T cells, they may not necessarily be identical to in vivo–differentiated effector T cells. For example, insights from work predating the transgenic TCR models showed that in vitro culture profoundly alters the repertoire of adhesion molecules expressed by T cells, which translates into progressively impaired in vivo trafficking. Indeed, large numbers of cells transferred after prolonged in vitro culture are trapped, e.g., in lung and liver (11). Alternatively, the impaired cells described by Gattinoni et al. (9) may well exert protective effector functions, but alone are not sufficient to provide persistent antitumor protection. These points must be stressed in order to avoid premature conclusions about the incompetence of highly differentiated effector CD8+ T cells. The possibility remains that some other cellular components essential for long-term control of tumor progression may have been missing from these cells. What could be missing? In order to investigate this, it seems necessary to elucidate how CD8+ T cells differentiate during acute and chronic immune responses. Despite the large number of studies regarding this issue (12–18), it remains difficult to precisely define the molecular features and roles of T cells at various stages of differentiation. Several in vivo models suggest that effector T cell function and long-term T cell persistence are ensured in part by different T cell subpopulations (i.e., by so-called effector and memory cells, respectively). This supports the notion that T cells at multiple differentiation stages are necessary to achieve long-term protection.

Subpopulations of effector T cells

Some subpopulations of effector T cells have limited functional and survival potential in vivo. In situations of extended and/or prolonged antigenic stimulation, effector T cells may become exhausted or (prematurely) senescent (Figure 1), which results in functional impairment and/or reduced cell survival. This has been observed during chronic viral infection in mice and humans, in TCR and cognate antigen double-transgenic mice, and in patients with HIV, hepatitis C, and melanoma (19–24). Despite considerable technical developments, we still lack appropriate methods for distinguishing effector, exhausted, and senescent cells (which all share most features of effector T cells), which may explain why discrepant roles are attributed to effector T cells. Clearly, we need to identify many more molecular features of T cells at various stages of differentiation in order to distinguish them and more precisely determine their roles in vital functions of T cell–mediated immunity. Relatively new approaches have allowed researchers to elucidate the replicative history of T cells. Human telomeres, more so than their murine counterparts, lend themselves to appropriate measurements (25). Another useful tool may be the quantitation of TCR rearrangement excision circles (TRECs), which act

as indicators of thymic output and relative peripheral T cell expansion (26). However, TREC levels are low in effector T cells and are often undetectable, which makes it unlikely that this approach will help to distinguish different subpopulations of effector T cells. Nevertheless, TREC analysis is useful for the distinction between naive and non-naive T cells. Finally, analyzing murine and human T cell responses at the level of individual (dominant) T cell clones may contribute significantly to our knowledge of T cell differentiation and competence. Comprehensive investigations using appropriate technologies will be instrumental in determining the key players among T cell populations responsible for successful ACT or active immunotherapy.

T cell selection and preparation for ACT

Gattinoni et al. also report very useful results using ACT with selected $CD8^+$ T cells either positive or negative for the lymph node homing receptor, CD62 ligand (CD62L) (9). Similar to the results from previous mouse experiments examining the role of effector T cells in protection against viral disease (27), effector T cells alone (CD62L-negative cells) showed low protective potential. CD62L-positive T cells, however, showed superior efficacy against melanoma following ACT. The higher therapeutic activity on a per-cell basis appears to be related to at least 2 intrinsic properties of CD62L-positive T cells: lymph node homing and in vivo expansion potential in the lymphopenic mouse. Future studies may address the questions of whether CD62L-positive T cells rapidly give rise to predominantly CD62L-negative (effector) T cell populations shortly after ACT and whether this is crucial for immunity. It would also be interesting to evaluate whether CD62L-mediated lymph node homing is required for protection and whether this may be a general feature of successful ACT. Lymph node homing may be specific for the applied mouse model (9), which appears to depend on tumor antigen cross-presentation primarily by DCs residing in lymph nodes. Is this representative of human tumors? Or do human tumors vary in their requirement for antigen cross-presentation?

For a comprehensive appreciation of the complexity of ACT, further emphasis must be given to the various strategies of patient conditioning (e.g., therapies to deplete lymphocytes in vivo prior to ACT), vaccination, and supporting treatment after ACT (e.g., high-dose IL-2 administration). In consideration of these factors, optimal therapeutic efficacy may depend on different T cell selection and preparation strategies.

In summary, it remains difficult to precisely define the optimal differentiation stages of T cells most suitable for ACT. In view of the well-known fact that prolonged culture in vitro is deleterious to T cells, a pragmatic strategy for human ACT is to keep the in vitro T cell expansion phase as short as possible. In addition, it is necessary to develop strategies to limit the loss of key cell subpopulations and cellular functions. The finding that IL-15 promotes proliferative and survival potential of $CD8^+$ T cells (9, 28) may be an important key to improving current ACT strategies for the treatment of cancer patients. With any luck, recombinant human IL-15 will soon be available and approved for clinical use so that clinical trials can rapidly clarify whether addition of IL-15 to T cell cultures, and/or patient treatment with IL-15, leads to improved therapeutic efficacy.

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