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Adoptive therapy with CD8+ T cells: it may get by with a little help from its friends

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The clinical goal of immunotherapy has traditionally been to provide either active or passive immunity against an infection or malignancy. In the active approach, antigen is administered (with or without some form of adjuvant) to initiate the generation within the host of a protective or therapeutic immune response. By contrast, in adoptive (or passive) cellular immunotherapy, the specific effectors of immunity are directly administered, bypassing the obstacles in the host that might prevent the generation of an effective response in vivo. Adoptive immunotherapy with CD8+ T cells permits the use of large numbers of effector T cell clones or lines of defined specificity and function, but has the disadvantage of requiring significant effort and technologic skill to isolate and expand such CTLs in vitro to the numbers needed to establish an effective in vivo response and successfully control the infection or tumor.

Adoptive T cell therapy is currently being investigated as an approach to treat both infectious (e.g., cytomegalovirus [CMV], HIV, and Epstein-Barr virus [EBV]) and malignant (e.g., melanoma and leukemia) diseases in humans (1–6). Preclinical and clinical studies have elucidated several issues that need to be addressed to improve the likelihood of success — including choosing appropriate target antigens, optimizing the methods for generating functional T cells in vitro, and providing requisite cytokines and/or proinflammatory signals to support the survival and effector functions of the T cells after infusion. In this issue of the *JCI*, Tuma and colleagues have used a murine model of immunity against the intracellular bacterium *Listeria monocytogenes* to evaluate a potential obstacle to T cell therapy, and demonstrate the utility of providing an inflammatory stimulus (CD40 activation) to rescue otherwise ineffective adoptively transferred CD8+ T cells (7). Their provocative findings are discussed below among the strategies currently being investigated to overcome current limitations of adoptive T cell therapy.

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Nonstandard abbreviations used: cytomegalovirus (CMV); Epstein-Barr virus (EBV); T cell receptor (TCR); antigen presenting cell (APC); activation-induced cell death (AICD).

Quantitative limitations of adoptive CD8+ T cell therapy

Perhaps the most basic limitation to providing effective adoptive CTL therapy is the need to generate sufficient numbers of CTLs in vitro for infusion. This is fundamentally a technical issue, requiring a greater understanding of the events associated with T cell activation and the optimization of various parameters of in vitro expansion (e.g., cytokines, T cell receptor [TCR] triggering signals, costimulatory signals, and feeder cells]. For example, recent studies have suggested that providing anti-apoptotic signals via ligation of the 4-1BB costimulatory molecule on CD8+ cells in addition to other costimulatory and TCR signals may greatly enhance the cell yield (8). What ultimately qualifies as a "sufficient" cell dose will depend upon multiple factors, including the antigen burden in vivo, the replicative ability of the transferred effector cells relative to the target, the effector function required, and the persistence and homing ability of the transferred CD8+ cells.

A closely related quantitative issue derives from the observation that in vitro expanded CTL clones administered in the context of high antigen burden often do not persist beyond a few days after infusion into patients (1, 2). Potentially this might be overcome by repetitive infusions of high doses of CD8+ T cells, but such an approach would be technically very demanding. Alternatively, this might be accomplished by administering IL-2 or CD4+ T helper cells concurrently with the CD8+ cells, which have been shown to prevent deletion or exhaustion of the infused CD8+ CTLs both in animal models and human clinical trials (1, 9–11). For example, in the treatment of EBV-induced lymphoma, transferred EBV-specific CD8+ CTLs were shown to persist for many months after the infusion of mixed CD4+/CD8+ lines (3). In addition to providing help to CD8+ effectors, CD4+ cells may enhance therapeutic activity via direct or indirect effector functions. Although the use of antigen-specific CD4+ T cells with CD8+ T cells represents an attractive therapeutic strategy to pursue, in many settings (such as HIV infection and established tumors) it may not be possible to isolate CD4+ T cells specific for relevant antigens, making it essential that alternative strategies to effectively maintain the CD8+ response also be explored.

Lastly, infused CD8+ T cells that do persist may still be prevented from replicating or even functioning in vivo by a host factor. Such a negative influence could be due to either active functional suppression (such as by tumor-produced factors or by T regulatory

cells), or the lack of an adequate niche within which the infused CTLs can replicate and expand. Although the size of the T cell compartment can be transiently increased, host homeostatic mechanisms ultimately normalize the size of the lymphocyte pool by balancing cell replication and cell death via multiple factors including cytokines and (self) antigen presentation (12). Thus, lymphodepletion has been observed to improve the persistence and therapeutic activity of subsequently transferred $CD8^+$ cells in murine models of adoptive therapy by creating an environment that promotes cell expansion (13, 14). Recently, Dudley and colleagues treated patients with advanced metastatic melanoma with infusions of ex vivo expanded CTLs and very high–dose IL-2 preceded by a conditioning regimen that caused significant lymphodepletion (5). Dramatic expansion and persistence of the infused CTLs with apparent retention of function were observed. Although clear antitumor effects were achieved, it remains unresolved if such an aggressive and potentially toxic approach (with respect to the extent of lymphodepletion and the dose of exogenous IL-2 administered to promote CTL function/proliferation) will prove necessary to maximize efficacy, particularly in settings of low rather than high antigen burden.

Qualitative limitations of infused T cells

The in vitro conditions for generating large numbers of antigen-specific CTLs in vitro for adoptive T cell therapy has the potential drawback of altering the functional capability of the $CD8^+$ T cells. There are countless protocols for stimulating, isolating, and expanding naive, memory, or effector CTLs ex vivo that each use a different combination of antigen presenting cells (APCs), cytokines, forms and concentrations of antigen, and durations of stimulation cycles. Although the ultimate goal of each of these in vitro methods is to somehow duplicate the result of a successful in vivo immune response and generate optimal effector CTLs that can survive and function after infusion, the CTLs could theoretically be "improperly programmed" during in vitro culture and expansion due to suboptimal or inappropriate stimulation conditions (e.g. inadequate or excessive provision of cytokines, costimulation, and/or TCR triggering). As a result, the CTLs could be deficient in effector functions such as cytolytic activity, deleted of high avidity $CD8^+$ cells (15), unable to sustain function in vivo, or programmed to undergo activation-induced cell death (AICD) following target recognition rather than becoming appropriately activated to survive and expand.

In the murine *Listeria* model described in this issue, antigen-specific CTLs infused into naive mice were able to provide protection from subsequent infection by the bacterium, but only within the first few days after infusion (7). Although the infused $CD8^+$ cells were still present a week after transfer, these cells no longer provided protection from infection, and, rather than expanding, were deleted in response to infection. These results suggest the $CD8^+$ cells had acquired a functional defect leading to AICD following target recognition. Tuma and colleagues further found that anti-CD40 monoclonal antibody administered to the host both before CTL infusion and at the time of bacterial infection a week later rescued the ability of the infused CTLs to control the infection, which they propose was due to "reprogramming" of the CTLs via an indirect non-specific inflammatory stimulus. Delivering a signal to APCs through CD40 has been shown to be important for the formation of memory $CD8^+$ cells following an otherwise apparently effective activation of $CD8^+$ T cells (16), and the reported results might reflect a nuance of the methods used to generate *Listeria*-specific effector cells. Unlike the in vitro systems employed in human studies, which typically employ dendritic cells matured with signals such as CD40 ligation as APCs, this study used peptide-pulsed spleen cells as stimulators which contain a variety of "professional" and "non-professional" APCs. Additionally, although the authors discount a role for a direct action of CD40 signaling in $CD8^+$ CTLs, recent evidence suggests that triggering the limited amount of CD40 expressed on $CD8^+$ T cells might be important for the generation of T cell memory (17). Thus, the generic role of proinflammatory signals, the specific role for CD40 signaling, the precise settings in which such signals might be necessary, and how to time such events with T cell transfer, all remain to be elucidated.

Target evasion as an obstacle

Even if large numbers of functional T cells are administered, adoptive T cell therapy may still be unsuccessful in controlling infection or malignancy, due to any one of multiple mechanisms of target evasion. For example, the kinetics of disease progression might overwhelm or outpace the immune response. This has contributed to establishing one of the basic principles of cancer immunotherapy, namely that success will most likely be achieved in a state of minimal residual disease (18).

Infectious or malignant diseases may still evade elimination even in the context of a small antigen burden by downregulating MHC expression — such as observed with CMV infection and melanoma (19, 20) — or by otherwise interfering with antigen processing and presentation. Targeted immune responses may be more specifically evaded by either mutation or loss of expression of the selected antigen. Such evasion has been well illustrated by the rapid evolution of strains of HIV resistant to endogenous T cell responses (21), and selection of target-antigen loss mutants following immunotherapy of melanoma (4, 22). Therapeutic success will require addressing these issues as part of the treatment design.

What can be done to improve $CD8^+$ T cell therapy?

With the above caveats, one might be prompted to pursue therapeutic avenues other than adoptive T cell transfer. However, the potential to successfully treat an infection or malignancy using highly specific effectors with minimal side effects remains too attractive to disregard. For this to evolve from an investigational tool to a viable form of therapy, improvements need to be made. First, choosing antigenic targets that are immutable and essential for survival of the infectious agent or malignant cell would reduce the potential for selecting antigen-loss variants. Currently, there are many strategies being employed that merge analysis of differential gene expression with antigen discovery, making it more likely that such desirable targets will be identified.

Areas with potential for immediate improvement include both the methods for in vitro generation of large numbers of highly active $CD8^+$ T cells

and modulation of the in vivo environment to more effectively support the transferred cells. Optimal generation of effector CTLs from naive precursors will likely require different conditions than stimulation of memory cells, or rescue of potentially anergized T cells reactive with tumor antigens and/or self-proteins. Current adoptive immunotherapy protocols often include IL-2 supplementation (or occasionally infusion of $CD4^+$ helper cells), to improve CTL effector survival and function, but other factors may also be useful to enhance the effect of adoptively transferred CTLs, either through direct interactions with the T cells themselves, or via indirect promotion of an inflammatory milieu. Examples of candidate factors include IL-12, IL-15, and CD40 stimulation (7, 23, 24). However, evaluation of such reagents will require carefully designed and analyzed studies, as suggested by the observations that IL-12 administration can be associated with significant clinical toxicities (25) and that CD40 stimulation can actually lead to the deletion of tumor-specific CTLs in certain circumstances (26).

Finally, combining insights from basic and preclinical studies with recent advances in clinical practice should lead to the development of novel and more effective treatment protocols. Advances in molecular immunology and genetic engineering are allowing the introduction of novel genes into T cells that can alter and/or enhance effector function. Examples of such molecules include chimeric immunoreceptors specific for tumor surface antigens that can target CTL function to tumor cells, chimeric GM-CSF/IL-2 receptors that can provide IL-2–independent autocrine growth signals, and receptors to improve CTL homing (27, 28). Such innovations hold great promise, and suggest that adoptive T cell therapy can become an effective and unique means to reliably treat infectious and malignant diseases.

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