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Review

Virus-specific T cells for therapy – Approaches, problems, solutions

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ABSTRACT

Adoptive T cell therapy is the transfer of T cells to a patient in order to combat disease. This procedure is mainly being used but not limited to the treatment of viral infections and malignancies including virus-associated tumors. Depending on the clinical context, the T cell donor may be the same patient or another donor, usually a healthy person. Recent research is centered on the use of antigen-specific T cells, but T cells of uncharacterized specificity can be successfully used in some clinical conditions where target antigens are not known. Depending on underlying scientific hypotheses and preferred technologies, the therapeutic T cells may be anything from monoclonal to highly polyclonal; they may be specific for one epitope, several epitopes from one antigen, or various antigens; they may have been selected during the preparation process for their specificity, their functional capacity, their survival and proliferation in vitro, or the expression of surface markers associated with desirable functional properties. In this minireview, we give a brief overview on selected approaches, problems and solutions in adoptive T cell therapy. We focus on an area where T cell therapy has been particularly successful but is still calling for improvement: herpesviral disease in patients after transplantation.

Rationale of T cell transfer

The principle of therapeutic T cell transfer, or adoptive T cell therapy, is at first sight of an appealing simplicity. The patient has developed viral disease, for example, because he or she lacks protection by appropriate virus-specific T cells, or lacks the capability to produce them in time. This defect will often be due to a pre-existing massive immunosuppression, in many cases caused by transplantation of blood stem cells or of a solid organ. To compensate for this deficiency, the patient is now receiving T cells that have been donated by a person with an intact T cell repertoire, or T cells that have been artificially generated or functionally restored in vitro. Upon transfer to the patient, specific cytotoxic CD8+ T cells will recognize the virus-infected cells, kill them and thereby prevent further spread of infection. In the case of a virus-associated malignancy, they will recognize viral oncogenes presented by the malignant cells, kill those cells and thus eliminate disease. Specific CD4+ T cells will help the CD8+ T cells to do their job by producing cytokines that will coactivate the CD8+ T cells and enhance antigen presentation by the infected target cells.

However, this simplified picture does not take into account several complicating aspects. For herpesviruses like Epstein-Barr virus (EBV) and cytomegalovirus (CMV) with complex, only partially understood lifestyles and a large array of antigens targeted by T cells (Adhikary et al., 2007; Hislop et al., 2007; Sylwester et al., 2005), it may be a challenge to identify those T cells that will prove protective in patients. The respective roles of CD8+ and CD4+ T cells may be less clear-cut than previously thought: CD4+ T cells may kill (Adhikary et al., 2006), and CD8+ T cells may provide help. Antigen-specific T cells just represent one important arm of an immune response. Even in viruses like EBV and CMV that tend to mobilize strong antigen-specific T cell responses, innate effector cells such as natural killer (NK) cells may be important antiviral effectors in their own right (Pappworth et al., 2007; Falk et al., 2002). Moreover, virus-specific antibody responses can protect from infection in the absence of T cells, as demonstrated in the murine CMV model (Klenovsek et al., 2007), although uncertainties remain about the efficiency of infusing CMV-specific immunoglobulins into patients with CMV disease (Sokos et al., 2002).

Diseases and patient backgrounds

There is a clear indication for virus-specific T cell transfer in patients who experience viral reactivations after allogeneic hematopoietic stem cell transplantation (allo-HSCT). Reactivations of herpesviruses such as CMV and EBV count as some of the most

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significant complications following allo-HSCT (Moss and Rickinson, 2005). These complications develop when there is a lack of virus-specific T cells (Reusser et al., 1991). CMV reactivations may lead to severe infection of organs such as the lung, gut or brain. A proportion of CMV reactivations is resistant to treatment with available antivirals such as ganciclovir and foscarnet, and these drugs have significant side effects such as neutropenia. Because CMV may reactivate from recipient tissue rather than from donor cells (Ljungman, 2007), the risk of CMV disease may be highest in the donor-negative/recipient-positive situation (Ljungman et al., 2003), because no CMV-specific effector cells are provided alongside the stem cell graft. In contrast to CMV, EBV reactivation may drive malignant cell growth in patients after HSCT, a condition called post-transplant lymphoproliferative disease (PTLD). The malignant cells are highly immunogenic, generally express several immunodominant EBV antigens, and thus should be easy targets of T cell therapy. Both EBV (in the form of infected B cells) and EBV-specific T cells travel from the donor to the patient, whereas the patient's own EBV-infected B cells are likely to be eliminated at the time of transplantation; this is the likely reason why the EBV-positive donor status, and a high B to T cell ratio in the transplant, are major factors predisposing to EBV disease (Moss and Rickinson, 2005). In order to ensure compatibility with the donor-derived immune system that is being reconstituted in allo-HSCT patients, virus-specific T cells traditionally need to be prepared from the same donor. For EBV and CMV, this is generally feasible when the donor is a virus carrier who has immunity to the virus, but poses a significant problem when the donor is virus-negative. This problem is of increasing relevance for CMV: whereas most adult donors worldwide can be expected to be EBV-positive, there is an increasing proportion of CMV-negative persons especially in the Northern hemisphere.

A different situation exists in recipients of a solid organ transplant (SOT) who develop CMV or EBV disease. For both CMV and EBV, virus-negative recipients who receive a positive graft or are infected later are predisposed to viral reactivation (Cox et al., 1995; Limaye, 2002). In spite of ongoing immunosuppression, it is possible to prepare virus-specific T cells from patients after SOT and to achieve clinical effects after T cell transfer (Khanna et al., 1999; Comoli et al., 2002; Brestrich et al., 2009), although the preparation of virus-specific T cells is more difficult than from healthy donors. This problem has been circumvented by using third party donor-derived T cells for therapy of EBV disease after SOT (Haque et al., 2007), an approach that proved unexpectedly safe and produced responses in a majority of patients.

Complexity and clonality of therapeutic T cells

T cells of very different degrees of complexity have been used in therapeutic T cell transfer, especially as far as CMV is concerned. Whereas it should be easier to precisely assess the specificity and non-toxicity of T cells of low clonality and complexity, their therapeutic efficacy might be lower, either because a suboptimal type of T cell has been unknowingly chosen, or because a combination of different T cells is required for optimal effect. One extreme case may be exemplified by the transfer of unpurified donor lymphocytes, a procedure of excellent clinical efficacy to prevent relapse of chronic myelogenous leukemia (Kolb et al., 1990), which has become a standard clinical procedure. The other extreme is represented by the first demonstration of antigen-specific T cell transfer to human patients. Here, one or a few monoclonal CMV-specific CD8+ T cell cultures prepared from their CMV-positive allograft donors were transferred to patients after HSCT (Riddell et al., 1992; Walter et al., 1995). Interestingly, the authors observed that the presence of CMV-specific CD4+ cells, which were not part of the transferred preparation, supported maintenance of the therapeutically transferred cells in patients. Thus, more recent strategies have generally sought to achieve a more complex composition of T cells by using complex antigen formulations and to include CD4+ T cells (Peggs et al., 2003). Cultures mainly containing CD4+ T cells have been used for therapy as well, a strategy that, in turn, favored the co-expansion of CD8+ T cells in patients (Einsele et al., 2002).

For EBV, efforts to produce specific T cells for therapeutic purposes have been nearly exclusively based on one well-established but complex culture system. In this system, EBV-specific T cells are selectively expanded from PBMCs by several weeks of cocultivation with an autologous EBV-transformed B cell line. This system has proved very successful in preventing or even combating EBV-associated PTLD in more than 100 patients overall (Rooney et al., 1995, 1998; Heslop et al., 2010). Although the authors calculate costs of only $6000 per T cell preparation (Heslop et al., 2010), its implementation according to current European regulations for clinical products might be more expensive, as this procedure requires up to 12 weeks of cell processing time. Because the EBV-transformed B lymphoblastoid cell lines (LCLs) used to stimulate EBV-specific T cells express at least 9–10 immunogenic EBV proteins potentially containing hundreds of T cell epitopes, the highly polyclonal nature of the resulting T cell preparations should be expected. However, it appears that single epitope specificities may sometimes dominate such T cell cultures, which could explain the observation that a single amino acid polymorphism in an EBV antigen rendered a patient's PTLD refractory to treatment by T cell transfer (Gottschalk et al., 2001). Although the T cell cultures contain variable proportions of CD4+ T cells (Heslop et al., 2010), it is still uncertain whether EBV-specific CD4+ T cells contribute to the therapeutic effects observed (Adhikary et al., 2007).

From EBV to EBV vectors

In order to take advantage of the benefits of the clinically highly successful EBV system for producing T cells with specificities other than EBV, we have established transgenic variants of EBV, called mini-EBVs, coding for heterologous proteins such as the CMV proteins pp65 or IE-1. In fact, we could show that mini-EBV-transformed B cells could be used to expand T cell lines that contained high proportions of CMV-specific CD8+ T cells (Moosmann et al., 2002) and substantial numbers of CMV-specific CD4+ T cells (Wiesner et al., 2005). This system was also useful for the characterization of previously unknown T cell epitopes (Wiesner et al., 2005). Currently, we are employing IE-1-expressing mini-EBV-transformed B cells in order to characterize the CD8+ and CD4+ T cell response to the CMV antigen IE-1. Preliminary results indicate that most CMV carriers harbor a numerically small but considerably diverse CD4+ T cell response to IE-1 (Ameres et al., unpublished), which appears particularly interesting because IE-1-specific T cell reactivity has been associated with protection from CMV disease (Bunde et al., 2005). We also constructed even smaller EBV vectors incapable of B cell transformation (Hettich et al., 2006) and found that these micro-/nano-EBV vectors nontheless mediate efficient antigen presentation to T cells (Ameres et al., unpublished). Thus, recombinant EBV technology offers promising possibilities for future immunotherapeutic applications. In a broader perspective, EBV- and mini-EBV-transformed B cells exemplify the potency of human activated B cells as antigen-presenting cells and immunotherapeutic tools (von Bergwelt-Baildon et al., 2002; Zentz et al., 2007; Wiesner et al., 2008).