γδ T Cells: A New Frontier for Immunotherapy?

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ABSTRACT

The use of cytolytic effector cells as therapy for malignant disease has been a central focus of basic and clinical research for nearly 2 decades. Since the original descriptions of in vitro lymphocyte-mediated cytotoxicity against human tumor cells, there have been numerous attempts to exploit such observations for therapeutic use, with decidedly mixed results. Most studies have focused on the role of either natural killer cells or cytotoxic CD8⁺ T cells as the primary mediators of antitumor cytotoxicity, and until recently little attention has been paid to the role of γδ T cells in this capacity. This is partially due to a lack of understanding of the mechanisms of γδ T-cell immune responses to tumors, as well as the practical problem of obtaining a sufficient number of γδ T cells for clinical-scale administration. In this article, we discuss the biological and clinical rationale for developing γδ T-cell-based immunotherapies for the treatment of a variety of malignant conditions. It is our view that infusing supraphysiological numbers of tumor-reactive γδ T cells—either in the autologous or allogeneic setting—might be used to restore or augment innate immune responses against malignancies. Accordingly, we will also discuss how we and others are working to overcome some of the practical limitations that have so far limited the direct clinical delivery of highly purified human γδ T cells for the treatment of both hematologic and solid tumors.

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KEY WORDS
γδ T cells • Immunotherapy • Cell therapy • Innate immunity

INTRODUCTION

The transfer of cytolytic effector cells into tumor-bearing hosts with the intent to eradicate disease has been the focus of a great deal of basic and clinical research for nearly 2 decades [1-13]. Since the original descriptions of lymphocyte-mediated cytotoxicity against human tumor cells in vitro, there have been numerous attempts to exploit such observations for therapeutic use in humans, with decidedly mixed results [5,14-16]. Clinical applications of adoptive cellular immunotherapy have included the treatment of patients with various malignancies (such as melanoma) by using interleukin (IL)-2–stimulated lymphokine-activated killer cells derived from autologous peripheral blood. Similarly, tumor-infiltrating lymphocytes, first isolated from primary tumors and subsequently cultured and expanded ex vivo, have also been administered clinically. Although historically a great deal of emphasis has been placed on the role of either natural killer (NK) cells or cytotoxic CD8⁺ αβ T cells as the primary mediators of antitumor cytotoxicity [17-24], until now little attention has been paid to the role of human γδ T cells in this capacity.

γδ T CELLS FORM PART OF THE INNATE IMMUNE DEFENSE AND ARE POTENT ANTITUMOR EFFECTORS

Whereas most mature T cells express the αβ T-cell receptor (TCR) heterodimer, a small proportion express an alternative γδ TCR heterodimer [25-28]. Unlike αβ T cells, which recognize specific processed peptide antigens presented on major histocompatibility complex (MHC) molecules by antigen-presenting cells, γδ T cells seem to directly recognize and respond to a variety of MHC-like stress-induced self-antigens expressed by malignant
cells [29-33]. Thus, γδ T cells can recognize malignant cells through less specific mechanisms that require no prior antigen exposure or priming, a function that is shared by other innate immune cells such as macrophages and NK cells [25]. Although γδ T cells comprise <10% of total peripheral blood T cells, they are present in substantially greater numbers within epithelial tissues such as skin, intestine, and lung [34-37], contrasting with αβ T cells, most of which either circulate in the peripheral blood or are resident in lymphoid organs.

The process by which γδ T cells recognize stressed or malignant cells is not completely understood. Although the TCR is involved in antigen recognition [38], the mechanism by which antigens are recognized by γδ T cells is fundamentally different from that for both αβ T cells and NK cells [25,39]. Although a detailed discussion on the biology of γδ T-cell recognition and the shaping of the γδ T-cell repertoire is beyond the scope of this article (several excellent reviews on this subject are available [39-42]), it is important to note that both genetic and extrinsic factors, such as environmental antigens, likely play a key role in shaping the γδ T-cell repertoire.

Several lines of evidence point to a role for γδ T cells in tumor immunosurveillance. It has recently been shown that mice lacking γδ T cells are highly susceptible to multiple regimens of cutaneous carcinogenesis [29]. In clinical studies, γδ T cells have been shown to infiltrate a variety of tumors, including lung cancer [43,44], renal cell carcinoma [45], seminoma [46], and breast cancer [47]. The most common circulating γδ T cells, ie, those expressing the Vγ9/Vδ2 TCR heterodimer (sometimes designated Vγ2Vδ2, because Vγ9 forms part of the Vγ2 gene family) [48], recognize several known tumor-associated ligands and cell lines. These include HSP-60 [49,50], Daudi Burkitt lymphoma [51,52], and gial cells [52]. Vγ9/Vδ2 γδ T cells recognize and lyse glioblastoma [53], neuroblastoma [54], multiple myeloma [55], and lung cancer [56]. CD30-restricted Vγ9Vδ2+ T cells have been isolated from patients with Hodgkin disease [57], and Vγ9/Vδ2 T cells recognize cells with increased mevalonate metabolites, which are overexpressed in hematologic malignancies and mammary carcinoma cells [58].

Vδ1+ T cells are less frequent, comprising up to 10% of all γδ T cells. They seem to recognize a different set of ligands and tumors, although there is some overlap with Vδ2+ cells. A high proportion of Vδ1+ γδ T cells appear in epithelial tumors from lung, breast, kidney, ovary, prostate, and colon that express the stress-induced antigens MICA and MICB [59], a nonclassical stress-related MHC antigen recognized by Vδ1+ cells [60]. Primary leukemias are also killed by γδ T cells. Duval et al. [61] showed that γδ T cells isolated from patients with leukemia expanded in IL-2-containing cultures to a greater degree than γδ T cells isolated from healthy controls. Vδ1+ and Vδ2+ T cells both expanded, and the Vδ1+ clones lysed the acute lymphoblastic leukemia (ALL) cell line NALM-6. Lamb et al. [62] later showed that Vδ1+ T cells proliferated when cultured with primary acute leukemia cells and became cytotoxic to the primary leukemia but did not lyse normal lymphocytes. In addition, Vδ1+ γδ T cells seem to recognize Epstein-Barr virus–transformed B cells [63], primary blasts obtained from patients with acute myeloid leukemia acute myeloid leukemia [64] and B-cell ALL [61], and lung cancer–derived cell lines [65]. However, the means by which γδ T cells recognize these targets are not yet understood.

SEVERAL PROPERTIES OF HUMAN γδ T CELLS MAKE THEM PARTICULARLY SUITABLE FOR INTENSIVE STUDY IN THE SETTING OF HEMATOPOIETIC STEM CELL TRANSPLANTATION

A number of in vitro and in vivo studies suggest that γδ T cells might be ideally suited for study specifically in the context of hematopoietic stem cell transplantation (HSCT). First, γδ T cells can mediate innate antitumor activity. Second, evidence suggests that γδ T cells might be capable of facilitating allogeneic engraftment. Moreover, it seems that γδ T cells likely to do not initiate graft-versus-host disease (GVHD). Despite these intriguing findings, however, few studies specifically address the role of γδ T cells in the setting of clinical HSCT.

Association between Allogeneic Graft γδ T-Cell Content and Disease-Free Survival

The first indication that γδ T cells might protect against disease relapse in bone marrow transplantation (BMT) patients was reported by Lamb et al. [66] in a study of patients undergoing allogeneic HSCT for ALL or AML. In this report, it was noted that several patients who received bone marrow grafts depleted of αβ T cells subsequently developed spontaneous increases in γδ T-cell numbers during the first year after HSCT. These patients were found to have a significant improvement in disease-free survival (DFS) when compared with similar-risk patients. It is interesting to note that the absolute increase in γδ T cells persisted in surviving patients for up to several years after transplantation. In a follow-up study, it was determined that a post-BMT absolute increase in γδ T cells was significantly associated with αβ T-cell depletion, because patients who received grafts that were T-cell depleted with OKT3, a pan T-cell monoclonal antibody, rarely showed an increase in γδ T cells after BMT (P = .05) [67]. Finally, Godder et al. [68] recently showed that the improved DFS of patients with increased γδ T cells is sustained over several years (Figure 1).
with the possible exception of the studies on patients with measured post-BMT increases in γδ T cells, is difficult to interpret because the γδ T-cell dose was not recorded and therefore was not included in the statistical analysis. Defined γδ T-cell dose-escalation studies in humans are essential to determine whether γδ T cells will protect against relapse.

**It Is Unlikely That γδ T Cells Initiate GVHD**

Both murine and human studies suggest that γδ T cells are not primary initiators of GVHD and may in fact modulate the GVHD activity of αβ T cells. Drobyski et al. [71] showed that large doses of IL-2-expanded γδ T cells could be infused into lethally irradiated MHC-disparate mice (C57BL/6 [H-2^b] α B10.BR [H-2^k] and C57BL/6 [H-2^b] α B6D2F1 [H-2^{bd}]) without causing GVHD. Ellison et al. [72] noted that γδ T cells were activated in the GVHD reaction but found no evidence that GVHD was initiated by γδ T cells. This work is agreement with later studies by Drobyski et al. [73], who showed that although activated γδ and naive αβ T cells exacerbated GVHD when infused together, delaying the infusion of αβ T cells by 2 weeks resulted in improved survival.

In human studies, Schilbach et al. [54] and Lamb et al. [62] found γδ T cells not to be substantially activated in the in vitro allogeneic mixed lymphocyte culture. Several post-BMT studies have shown transient increases in γδ T cells [74-76] but have not associated this finding with GVHD, although Tsuji et al. [77] found that γδ T cells could be recruited into lesions and activated by CD4+ αβ T cells. Several studies that compared outcomes of patients who received αβ T-cell-depleted grafts with those of patients who received pan T-cell-depleted grafts all showed a lower incidence of GVHD in the αβ T-cell-depleted group, thus suggesting that infusion of γδ T cells in the graft does not subject the patient to an increased risk of GVHD [69,70,78]. Whether γδ T cells are truly less likely to contribute to the development of GVHD remains untested. However, from the previous reasoning, it is both logical and rational to propose that in future studies, γδ T cells might indeed be introduced in the setting of allogeneic HSCT—specifically to provide an innate antitumor effect—yet represent only a minimal risk of causing GVHD.

**Animal Studies and Indirect Evidence from Human Allogeneic Transplantation Studies Suggest That γδ T Cells Can Also Facilitate Alloengraftment**

Blazar et al. [79], in a murine allogeneic transplantation model, found that donor γδ T cells facilitate the engraftment of T-cell-depleted donor bone marrow. When T-cell-depleted marrow from the severe combined immunodeficient mouse strain C.B17-scid/scid
(H2d, T10β) was supplemented with up to 3 × 10^6 γδ T cells, engraftment into sublethally irradiated B6 mice was significantly improved over B6 mice receiving TCD marrow alone. Drobyusk and Majewski [80] noted similar findings when C56BL/6(H2b) donor marrow was supplemented with γδ T cells prior to transplantation into B10.BR (H-2b) recipients. In addition, the γδ T cell dose necessary to facilitate engraftment did not result in lethal GVHD [71]. Neipp et al. [81] showed similar findings in a rat model in which lethally irradiated (Wistar Furth WF-RT1A) rats were reconstituted with 1 × 10^6 αβ T cell–depleted bone marrow. All animals engrafted with a mean of 92% ± 4% donor cells and no clinical evidence of GVHD. Studies comparing patients who received αβ T cell–depleted grafts with those receiving pan T cell–depleted grafts also show a positive association between the number of clonable γδ T cells in the graft and less time to engraftment [82,83].

**OBSTACLES REMAIN TO CLINICAL APPLICATION OF γδ T-CELL THERAPY, BUT THESE ARE BEING OVERCOME**

Given the previously described information, it stands to reason that it might be possible to develop clinical strategies whereby human γδ T cells are specifically introduced or incorporated as part of an allogeneic HSCT product when transplantation is performed for the treatment of various malignancies. Nevertheless, the difficulty of isolating suitable numbers of human γδ T cells given their relative infrequency in peripheral blood has remained a major obstacle to the development of clinical models to exploit the innate antitumor activity of human γδ T cells. In addition, human γδ T cells cannot readily be expanded. We and others have come to recognize that in vitro, standard culture methods used to expand human T cells are unsuitable for the expansion of γδ T cells because the strong T-cell mitogens often used can directly induce apoptosis in γδ T cells; they are extremely sensitive to activation-induced cell death [84-87].

The development of protocols for γδ T cell–based adoptive cellular immunotherapy has been seriously hampered by their sensitivity to activation-induced cell death, which has prevented the clinical-scale expansion of γδ T cells. In a series of recent publications, however, our laboratory has identified and characterized a CD2-mediated, IL-12–dependent signaling pathway that inhibits apoptosis in mitogen-stimulated human γδ T cells [87,88]. Our working model proposes that CD2-mediated, IL-12–dependent signals lead to the preferential expression of the IL-15 receptor α (IL-15Rα) chain over the IL-2Rα chain in γδ T cells. By our convention, these γδ T cells are referred to as “protected” γδ T cells. In contrast, γδ T cells that receive no CD2-mediated, IL-12–dependent signals (referred to as “unprotected” γδ T cells) persist in their expression of the IL-2Rα chain and, thus, remain exquisitely sensitive to apoptosis induced by IL-2 [85]. Responsiveness to IL-2 or IL-15 is determined by the respective expression of either the IL-2Rα or the IL-15Rα chain in association with the β chain and the common γ chains. Our model proposes that a coordinated downregulation of the IL-2Rα chain and a corresponding upregulation of the IL-15Rα chain occur as a consequence of CD2-mediated, IL-12–dependent signaling. Given the contrary effects of IL-2 and IL-15 on mitogen-stimulated γδ T cells, we propose that CD2-mediated signals, through the effects of IL-12, determine the fate of mitogen-stimulated γδ T cells by altering their responsiveness to IL-2 and IL-15 [88]. Indeed, γδ T cells that are induced to express the IL-15Rα chain (message and protein), which in turn can respond to IL-15, subsequently express substantially higher levels of message for bcl-2: this is likely important in the acquisition of an apoptosis-resistant phenotype. Although the biologic and antitumor characteristics of human γδ T cells expanded in this manner have not been completely characterized, initial studies are currently being performed to determine the in vivo efficacy and safety in a human/mouse xenograft model.

These findings are important for both practical and clinical reasons. First, by exploiting this signaling pathway, development of methods that permit the large-scale ex vivo expansion of viable, apoptosis-resistant human γδ T cells has been made possible. Moreover, expanded γδ T cells—whether derived from normal healthy donors or from cancer patients—retain significant innate, MHC-unrestricted cytotoxicity against a wide variety of human-derived tumor cell lines, including myeloma, leukemia, melanoma, non–small-cell lung carcinoma, hepatocellular carcinoma, and ovarian and breast carcinoma cell lines [55,87,89].

Future studies must be designed to determine whether infusion of supraphysiologic numbers of γδ T cells will restore or augment innate immune responses against selected malignant diseases and thus moderate disease progression or the likelihood of relapse after standard initial therapy. Allogeneic HSCT, for reasons discussed previously, may be the optimal setting to study the effects of γδ T-cell therapy. Donor-derived γδ T cells would be incorporated into the transplantation procedure as a donor lymphocyte infusion (DLI). DLI is sometimes used as nonspecific cellular therapy for disease relapse or as prophylaxis against relapse after allogeneic HSCT [90-96]. Because DLI is usually performed by delivery of unfractionated donor T-cell preparations consisting primarily of αβ T cells, severe GVHD is a common complication. Given the potentially lower risk for ini-
tiation of GVHD by γδ T cells, it may be possible to deliver donor-derived γδ T-cell DLI early after non-myeloablative allogeneic HSCT with a minimal risk of GVHD.

Alternatively, it has been shown that autologous tumor-reactive γδ T cells can be expanded from patients and that these expanded cells retain significant innate antitumor cytotoxicity in vitro. Therefore, it is possible that autologous tumor-reactive γδ T cells can first be obtained from a patient, expanded ex vivo, possibly cryopreserved, and then administered in supraphysiologic numbers at a subsequent point—likely in conjunction with other more standard therapies. Such a strategy would rely in large measure on the innate ability of γδ T cells to recognize and eradicate residual malignant disease. However, to rationally develop this model, several important questions must first be addressed, including determining the optimal point during a patient’s clinical course to collect, expand, and cryopreserve autologous γδ T cells. Similarly, it would be necessary to determine at what point reinfusion of expanded autologous γδ T cells might be performed to best exploit their innate antitumor activity.

Finally, recent studies have shown that pharmacologic therapy with aminobisphosphonate drugs can be used to activate and expand γδ T cells in vivo, thereby inducing a γδ T cell–mediated antitumor effect [55,97]. Dieli et al. [98] recently showed that zoledronic acid administration results in activation and proliferation of peripheral blood γδ T cells in several patients with solid tumors. In addition, Kunzmann et al. [55] documented a measurable γδ T cell–mediated antiplasma cell effect in bone marrow cultures derived from multiple-myeloma patients. This effect is lost when γδ T cells are removed from culture. Wilhelm et al. [97] later showed that IL-2 in combination with pamidronate was effective in inducing a measurable reduction of multiple myeloma and non-Hodgkin lymphoma in 3 of 5 patients in whom γδ T cells numbers were increased. In addition, γδ T cell cytokine (interferon γ, tumor necrosis factor α, and IL-6) production was increased after a single infusion of pamidronate. It is therefore logical that aminobisphosphonate and γδ T-cell therapy may be an effective immunotherapeutic approach to sensitive tumors such as myeloma, lymphoma, and breast cancer.

CONCLUSIONS AND FUTURE DIRECTIONS

Learning to exploit the innate antitumor properties of human γδ T cells—particularly as a complement to the more classic adaptive immune responses—may allow us to improve our current abilities to treat a variety of malignant hematologic diseases for which allogeneic HSCT is commonly used. However, given the biology of γδ T cells—particularly their innate ability to recognize and kill malignancies of epithelial origin—the exciting prospect of extending allogeneic HSCT to the treatment of diseases other than those routinely approached with allogeneic HSCT must now also be considered.

Barriers to the use of γδ T cells for such therapy—specifically, their relative scarcity and tendency to undergo activation-induced cell death—are now being overcome. Recent studies have shown that large numbers of γδ T cells that retain significant antitumor activity can be produced with methods that are easily adaptable to current cell-processing regulatory requirements. Techniques are currently being developed for cyclic guanosine monophosphate–compatible clinical-scale ex vivo expansion of γδ T cells. The first clinical trials are expected within the next 6 to 12 months.

For these reasons, we envision that in the setting of nonmyeloablative allogeneic HSCT, it will eventually become possible to specifically transfer tumor-reactive donor-derived γδ T cells as part of the transplantation strategy for the treatment of a variety of hematolymphoid and epithelial-derived malignancies. Particularly in the setting of nonmyeloablative HSCT, we predict that the scheduled delivery of donor-derived γδ T-cell DLI will be associated with minimal to no GVHD, a lower risk of graft rejection, and a measurably lower risk of relapse, thus translating into a corresponding increase in long-term DFS and overall survival in patients undergoing such therapy.

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