Cytotoxic and Regulatory Properties of Circulating Vδ1+ γδ T Cells: A New Player on the Cell Therapy Field?

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Exploration of cancer immunotherapy strategies that incorporate γδ T cells as primary mediators of antitumor immunity are just beginning to be explored and with a primary focus on the use of manufactured phosphoantigen-stimulated Vγ9Vδ2 T cells. Increasing evidence, however, supports a critical role for Vδ1+ γδ T cells, a minor subset in peripheral blood with distinct innate recognition properties that possess powerful tumoricidal activity. They are activated by a host of ligands including stress-induced self-antigens, glycolipids presented by CD1c/d, and potentially many others that currently remain unidentified. In contrast to Vγ9Vδ2 T cells, tumor-reactive Vδ1+ T cells are not as susceptible to activation-induced cell death and can persist in the circulation for many years, potentially offering durable immunity to some cancers. In addition, specific populations of Vδ1+ T cells can also exhibit immunosuppressive and regulatory properties, a function that can also be exploited for therapeutic purposes. This review explores the biology, function, manufacturing strategies, and potential therapeutic role of Vδ1+ T cells. We also discuss clinical experience with Vδ1+ T cells in the setting of cancer, as well as the potential of and barriers to the development of Vδ1+ T cell-based adoptive cell therapy strategies.

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INTRODUCTION

The study of cancer immunology and immune therapy has been a significant focus of basic and clinical research since early discoveries of tumor antigens and adoptive immunity.[1–3] As various lymphocyte subsets have been identified, more specific strategies for cancer immunotherapy began to develop, most of which continue to focus on natural killer (NK) cells or cytotoxic T lymphocytes (CTL) as the primary mediators of antitumor immunity.[4–11] In addition, these cell types can easily be isolated, expanded, and activated *ex vivo* leading to manufacturing strategies that have shown promise in effecting durable remissions for a growing number of cancers. The contribution of γδ T cells, a minor T cell subset with distinct innate recognition properties, has not been explored until recently.

Most mature T cells express the αβ T cell receptor (TCR), reside in the secondary lymphoid organs, and function primarily in adaptive immune responses. CD3+γδ+ T cells are a relatively rare immune effector population in peripheral blood (4–10% of T cells) but are substantially enriched in epithelial tissues,[12] where they function as primary responders by recognizing intact structures such as stress-associated proteins, heat shock proteins, and lipids[12,13] in a classical MHC-unrestricted manner.[12,14] Here, they also manifest lytic activity and proinflammatory cytokine secretion. These cells are now known to play a critical role in tumor immunosurveillance[15–18] and in the immune response to cancer.[19–24] In many instances, γδ T cells that are cytotoxic to a specific tumor type will cross react with other tumors but not with the tumor's nontransformed counterpart.[22,23,25]

Activating ligands for γδ T cells as well as the process by which they recognize stressed or malignant cells are complex and incompletely understood, but are fundamentally different from both γδ T cells and NK cells.[13,26–28] The most prevalent circulating population of γδ T cells expresses the Vγ9Vδ2 TCR that uniquely responds to nonpeptide alkylphosphates, such as isopentenyl pyrophosphate (IPP), a product of the mevalonate pathway of isoprenoid biosynthesis[29] that is dysregulated in tumor cells and upregulated in individuals exposed to bone-strengthening aminobisphosphonate (N-BP) compounds, such as Zoledronate and Pamidronate. Vδ2+ T cells have antitumor effector function, are relatively simple to manufacture in large numbers, and have been employed in early phase autologous cell therapy trials against solid tumors with mixed results.[30,31] Wider implementation of Vγ9Vδ2+ T cell therapy protocols has been hampered by uneven responses to *ex vivo* stimulation and the strong propensity of this population to undergo activation-induced cell death (AICD), severely limiting the persistence of effector function.[25,32,33]

Increasing evidence supports a critical role for a particular subset of γδ T cells that bears the Vδ1+ TCR in tumor

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immunosurveillance. Vδ1+ T cells are a minor subset with the distinct innate recognition and regulatory properties that possess powerful tumoricidal activity. Unlike Vδ2+ cells, they do not preferentially pair with a specific Vγ chain, and are not activated by IPP or N-BP.[34–36] Vδ1+ T cells are activated by a host of ligands including stress-induced self-antigens, glycolipids presented by CD1c, and others as discussed in detail below.[37–39] In contrast to Vδ2+ T cells, the Vδ1+ T cell population is not as susceptible to AICD, and tumor-reactive Vδ1+ T cells can persist in the circulation for many years.[40,41] The cytotoxic function of Vδ1+ T cells has been described for lymphoid and myeloid malignancies,[42–47] neuroblastoma,[48] and cancers of the lungs, colon, and pancreas.[49–51] Primary myeloid and lymphoid leukemias directly activate Vδ1+ T cells[43–45] and generate effector function against both primary leukemia and cultured leukemia cell lines. Specific populations of Vδ1+ T cells can also exhibit immunosuppressive and regulatory properties, a function which is discussed at greater length below.

This review explores the biology, function, manufacturing strategies, and potential therapeutic role of blood-derived/circulating Vδ1+ T cells. For a discussion of general aspects of γδ T cell biology, the reader is directed to several excellent contemporaneous reviews.[52–55] We also discuss clinical experience with Vδ1+ T cells in the setting of cancer, and the potential of and barriers to the development of Vδ1+ T cell-based adoptive cell therapy strategies.

LIGAND RECOGNITION BY Vδ1 T CELLS

Ligand recognition by Vδ1+ T cells constitutes largely uncharted territory for those exploring options to bring these cells into the clinical arena. While some methods have been developed to expand these cells *in vitro* (discussed below), further identification of ligands recognized by circulating Vδ1 would greatly aid in the generation of Vδ1+ T cell cultures to the scale and desired immunophenotype required for therapeutic use.

Although rare instances of CD4 or CD8 coexpression have been reported for Vδ1+ T cells,[56,57] their developmental program generally does not include the expression of CD4 or CD8 nor require the extensive proliferation or multiple TCR recombination events that are characteristic of αβ T cells. The diversity of the γδ TCR CDR3 region length suggests a broad pattern of ligand recognition not constrained to specific presentation, setting it apart from the αβTCR. The γδ T cell repertoire is shaped throughout the life; while the TCR J region is diverse in infants, it is significantly restricted as we age.[58] Germline-derived elements and combinations of the TCR V, D, and J segment of both γ and δ chains encode innate recognition of both proteins and nonproteins that include endogenous and synthetic phosphoantigens,[13,29,59–62] heat-shock proteins,[63–65] and stress-associated antigens.[42,66] Most γδ T cells (as well as NK cells and αβCD8+ T cells) also express NKG2D, a C-type, lectin-like homodimeric activating receptor that functions as a ligand for MHC class-I like proteins, such as MIC-A/B and the UL-16 binding proteins that are often upregulated on malignant cells.[67–69] Vδ1+ T cells are activated by these stress-induced self-antigens that are often constitutively expressed by solid tumors as well as some leukemias and lymphomas.[42,46,47,66,70–72] In particular, Vδ1+ T cells recognize MIC-A/B[66,73] induced by oxidative stress,[74] thus explaining the increased prevalence of Vδ1+ tumor-infiltrating lymphocytes (TIL) in MICA/B expressing tumors.[42] Recent elucidation of the crystal structure of a MIC-reactive Vδ1 TCR suggests sequential recognition of MIC by TCR and NKG2D.[75] Indeed, the presence of the NKG2D receptor on Vδ1+ and Vδ2+ (and most other known) γδ T cell subsets is critical for their role in cytotoxicity against various cancers.[67] Upon target recognition, Vδ1+ T cell-mediated killing is via perforin and granzymes *via* similar mechanisms to those of Vδ2+ T cells.

Interestingly, some Vδ1+ T cell lines recognize CD1c.[37,76,77] Furthermore, upon sensing glycolipids presented by CD1c on the surface of immature dendritic cells, Vδ1+ T cells could induce DC to mature and produce IL-12.[38] While there have also been some past reports of blood-derived Vδ1 cell recognition of lipid-based antigens presented by CD1d,[78–80] two groups recently took this one step farther by elucidating crystal structures of Vδ1 TCR bound to CD1d presenting two different ligands.[81,82] Uldrich *et al.*[81] investigated the molecular basis for the interaction of Vδ1 TCR with CD1d bound to α-GalCer, reporting that CD1d binds TCR mainly through the CDR1δ loop, with antigen specificity dictated by the CDR3γ loop. While there was substantial interdonor variability in the extent of lipid antigen reactivity, this finding is nonetheless of great interest. The therapeutic potential of α-GalCer as the classical ligand for Type I NKT cells has been recently tested in clinical trials to treat patients with advanced nonsmall cell lung cancer,[83] diverse head and neck cancers[84] and asymptomatic myeloma (for the latter in combination with lenalidomide).[85] Treatments were well tolerated and responses promising thus may translate to Vδ1 therapies incorporating α-GalCer. Luoma *et al.*[82] investigated Vδ1 TCR interaction with the self-ligand sulfatide and CD1d using blood-derived Vδ1 T cell clones; CD1d binding was mediated via the CDR loops of the δ-chain.

While Vδ2 ligands are fairly well defined and can thus be used to manipulate Vδ2 both *in vitro* and *in vivo* (see introduction), specific Vδ1+ TCR ligands are still largely unknown, yet some interesting leads have been uncovered. The abovementioned studies suggest the distinct possibility of lipid-based antigens.[82] Also, there is an intriguing Vδ1+ T cell predominance in the blood of African adults[86]; while the evolutionary significance thereof has yet to be explained, further exploration could unlock ways to preferentially expand and manipulate Vδ1+ T cells. Qi *et al.*[87] took steps in this direction, capitalizing on the identification of MICA as a Vδ1 ligand and selectively expanding cytotoxic Vδ1+ T cells *in vitro* via immobilized recombinant MICA. To augment Vδ1+ T cell targeting of lymphoid leukemia, Correia et al used IL-2 or IL-15 in conjunction with TCR stimulation to induce expression of natural cytotoxicity receptors NKp30, NKp44 and NKp46.[88] Also, upregulation of known ligands on targets can also be used to enhance Vδ1+ T cell cytotoxicity.[47]

Migration of Vδ1 cells into tumors has been described, yet only a few studies have focused on chemokine receptors responsible for these homing abilities. While chemokine (CXC) receptor (CXCR)-1 was found to be strongly and chemokine C-C motif receptor (CCR)-5 weakly expressed on peripheral Vδ1 cells, Vδ2 cells expressed comparatively less CXCR1 and more CCR5.[89] CCR5 expression is associated with Th1 polarization and IFNγ production and, on primary CD4+ T cells, decreases in the absence of

IL-2 or when cells are activated via CD3 and CD28 stimulation,[90] whereas it may increase in pathological contexts such as HIV.[91] Expression of CXCR1 suggests IL-8 responsiveness; since IL-8 is present in the tumor microenvironment and associated with advanced disease (reviewed in ref. 92), this could be a mechanism by which Vδ1+ T cells home to tumors. In another study, Vδ1+ T cells expanded from peripheral blood via antibody stimulation expressed more CCR4 and CCR8 than their Vδ2 counterparts[93]; moreover, these cells migrated preferentially toward CCL17 and CCL22, chemokines that serve as ligands for CCR4 (both) and CCR8 (CCL17) and are expressed by lymphoma cell lines as well as other tumor types.[93] Devaud *et al.*[94] found that CMV-reactive Vδ2-negative T cell clones (not necessarily Vδ1) expanded *in vitro* expressed CCR3, which was necessary for migration into and antitumor activity against xenograft HT29 colon carcinoma tumors that express factors such as IL-8, MIP-1δ, MIP-3α, and monocyte chemoattractant protein 4. Notably, CCR3 levels were maintained throughout activation and expansion of the clones.[94] More recently, Lança *et al.*[95] identified CCR2 on Vδ1 but not Vδ2 cells; CCR2 expression enabled migration to CCL2, a cytokine upregulated in oral squamous cell carcinoma, breast cancer, and prostate cancer. While these studies have provided a crucial first glimpse into how chemokines and chemokine receptor expression influence the migration of Vδ1 cells in the context of cancer, there is much room for further exploration. It will be important to document the impact of various *ex vivo* culturing methods on the expression of chemokine receptors critical for homing to the tumor types targeted by Vδ1 immunotherapy.

Vδ1+ T CELLS ARE POTENT ANTICANCER CELLS

The earliest indication of leukemia surveillance by γδ T cells was reported by Lamb[40] and Godder,[41] who showed a significant improvement in risk-adjusted 5–10 year disease-free survival (DFS) in patients with acute lymphoblastic leukemia (ALL) or acute myeloid leukemia (AML) who had received αβ T-cell depleted (TCD) allogeneic bone marrow grafts. Following bone marrow transplant (BMT), ~28% of these patients subsequently showed early homeostatic reconstitution of donor-derived Vδ1+ T cells up to 100× normally seen in the circulation[40] that persisted for several years, a finding that was also shown to be significantly associated with the receipt of αβ T cell depleted marrow.[96]

Fujishima[97] also reported peripheral expansion of Vδ1+ T cells in BMT patients. These cells, which show a clonally restricted δ1 CDR3, recognize EBV-transformed B cells, expand both *in vitro* and *in vivo,* and are also long-lived.[97] Dominant populations of circulating clonally-restricted Vδ1+ γδ T cells have also been described in children presenting with a new diagnosis of ALL.[98] Coculture of third party αβTCD mononuclear cells (MNC) with leukemic blasts from these patients grew a dominant population of Vδ1+ T cells that were cytotoxic to both the patients' primary blasts, ALL cell lines, and third-party ALL but not to normal lymphocytes.

Knight[99] described a series of BMT patients that developed a significant long-term expansion of a circulating and clonally restricted Vδ1+ T cell population associated with cytomegalovirus (CMV) reactivation during posttransplant recovery. CMV infection has also been shown to stimulate Vδ1+ T cells in solid organ transplant patients with Vδ1+ T cell proliferation increasing and decreasing in response to viral load. CMV-responsive Vδ1+ T cells cross-react with tumor cell lines that show no CMV infection or residues.[100–102] Although the mechanism of the observed cross-reactivity has not been elucidated for Vδ1+ T cells, Wilcox has described a Vγ4Vδ5 T cell clone that binds the endothelial protein C receptor expressed on epithelial tumors and endothelial cells targeted by CMV.[103] Conversely, CMV infection can also sequester NKG2D ligands resulting in decreased tumor immunogenicity.[104–107] Taken together, these findings suggest a multifaceted association between CMV recognition and antitumor immunity that warrants further study.

Circulating Vδ1+ T cells have been associated with nonprogression in low risk B-CLL patients and could kill autologous targets *in vitro*, with killing linked to ULBP3 expression on leukemia cells.[47] In addition, the same group showed that low-grade non-Hodgkin lymphoma (NHL) patients with high Vδ1+ T cell counts and elevated serum IL-4 experienced stable disease at 1 year follow-up, compared to those with lower IL-4 and Vδ1+ T cell levels.[46] Presumably, these Vδ1+ T cells expanded in response to UL-16 binding proteins (ULBPs) 2 and/or 3 expressed by NHL.[46] Vδ1+ T cells, but not Vδ2+ T cells, infiltrated ULBP-positive lymph nodes of NHL patients.[46]

In addition to hematopoietic malignancies, Vδ1+ T cells are exquisitely responsive and cytotoxic to neuroblastoma. After initial findings of significant cytotoxic activity of peripheral blood γδ T cells against human neuroblastoma cell lines, Schilbach[48,108] showed that TH1 cytokines are downregulated and tumor growth-promoting factors (ANG, VEGF, EGF, and IGF-I) upregulated in Vδ2+ T cells cultured in the presence of neuroblastoma. In contrast, Vδ1+ T cells cultured with the same tumor showed decreased production of tumor-promoting cytokines and TGF-β while concurrently upregulating TNF-α, TNF-β, MCP-1 and MCP-2 and maintaining IL-2 production.[48]

Examination of TIL from other solid tumors also supports Vδ1+ T cell response to malignancy, especially in epithelial tumors. Vδ1+ T cells isolated from the TIL of colon tumors were cytotoxic against both autologous and allogeneic epithelial tumor cells.[51] Both Vδ1+ and Vδ2+ T cell subsets are components of TIL isolated from melanoma[109]; when cultured, these cells do not appear to be functionally impaired as assessed by cytotoxic activity and production of IFNγ and TNFα. Interestingly, all cultured melanoma TIL-derived Vδ1+ T cell lines killed A375 cells, whereas only two of eight Vδ2+ T cell lines showed significant cytotoxicity.[109] In addition, Vδ2+ T cell-mediated cytotoxicity also required Zoledronic acid treatment of tumor targets to kill effectively, a finding separately reported by Nishio.[110]

Vδ1+ T CELLS ALSO EXHIBIT REGULATORY FUNCTIONS

While Vδ1+ T cells clearly exhibit potent antitumor activity, paradoxically, recent reports describe their potential regulatory function in the tumor microenvironment. Peng[111] observed Vδ1+ T cells with regulatory properties after culturing TIL obtained from breast tumors. A follow-up study that examined relationships between breast cancer TIL phenotypes and patient survival suggested that the frequency of infiltrating γδ T cells was a significant

predictor of negative outcome.[112] Cultured γδ TIL-derived regulatory cells did not express classical regulatory markers CD25 and FoxP3 nor was suppressive activity mediated by IL-10 or TGFβ. Interestingly, regulatory activity could be reversed via TLR8 signaling.[111,113] While these studies show regulatory capacity of Vδ1+ T cell cultures derived from TIL, a direct role for γδ T cell TIL in disease pathogenesis was not determined. Furthermore, specific Vδ subset phenotypes were not assessed in the primary tumor. These findings are also complicated by a more recent study describing regulatory properties for Vδ2+ TIL,[114] cells that may be lost due to AICD and therefore escape isolation and further study. Indeed, TIL cultures can be driven to Vδ1+ or Vδ2+ predominance depending on the conditions applied in the culture.[48,115]

Hua[116] showed that a classical regulatory phenotype could be induced in blood-derived Vδ1+ T cells by stimulation via plate-bound anti-Vδ1 antibody, promoting expression of regulatory markers FoxP3, CD25, CTLA-4, and corresponding suppression of CD4+ T cell proliferation. Moreover, TGFβ1 production by Vδ1+ T cells fed into a positive feedback loop, sustaining FoxP3 expression; these cells also produced the anti-inflammatory cytokine IL-10.[116]

In contrast, Vδ1+ T cells with an effector phenotype have been derived from melanoma. Cordova[109] cultured polyclonal Vδ1 TIL lines that secrete TNFα, IFNγ, and kill melanoma cell lines. Similar findings for cultured Vδ1+ TIL from metastatic melanoma were reported by Donia.[117] These inconsistencies might result from the differential infiltration of clones with various Vγ pairings that become activated in the context of different cancers. This calls into question the degree to which *in vitro* culture conditions can convincingly replicate the tumor microenvironment. Furthermore, naturally occurring Vδ1+ T cell migration to epithelial tissues may also influence TIL composition and function in the tumor microenvironment of melanoma compared to that observed in carcinomas.

CLINICAL-SCALE MANUFACTURING OF Vδ1+ T CELLS FOR THERAPEUTIC APPLICATIONS: A WORK IN PROGRESS

As discussed above, several investigators have developed procedures and trials for culturing γδ T cells for therapeutic use based on their responsiveness to bisphosphonate drugs, many of which are approved in the United States and Europe for osteoporosis and prevention of bone metastases in cancer patients. Strategies that employ good manufacturing practice (GMP)-approvable cell culture methods and pharmaceutical-grade reagents have been recently reviewed by Fournie,[118] and are easily translated for use in both allogeneic and autologous therapies. At issue, however, is the finding that both N-BP and phosphoantigen-mediated γδ T cell stimulation expands only the Vγ9Vδ2 γδ T cell subset, and thus does not deliver the potential therapeutic benefit of an expanded Vδ1+ population; furthermore, long-term persistence is minimal and difficult to achieve.[16,48]

To date, there has not been a single clinical study in which Vδ1+ γδ T cells have been specifically introduced as autologous or allogeneic cell therapy. Expansion techniques for Vδ1+ T cells remain small scale and laboratory-based although, with modification of reagents and purification techniques, some may be adaptable to clinical-scale cell manufacturing strategies. Lopez[119,120] was the first to develop a pan-γδ T cell expansion strategy, taking the advantage of a CD2-initiated signaling pathway that induces a coordinated down-regulation of the IL-2Rα chain and a corresponding upregulation of the IL-15Rα chain. The γδ T cells stimulated in this manner express 10-fold higher levels of message for *bcl*-2 resulting in an inhibition of apoptosis, thereby overcoming γδ T cell sensitivity to AICD while retaining potent innate antitumor activity against a wide variety of human hematopoietic and solid primary tumors and cell lines.[119,120] This method expands peripheral blood γδ T cells regardless of phenotype and is adaptable to clinical scale use.

Several investigators have taken the advantage of Vδ2 sensitivity to AICD, exposing γδ T cells to powerful plant mitogens and thereby generating a predominant Vδ1+ T cell population in culture. Schilbach *et al.* purified blood-derived γδ T cells by immunomagnetic selection followed by stimulation of purified cells with PHA and IL-2 in culture. Addition of pamidronate stimulated the Vδ2 population, which was subsequently lost from culture and resulted in outgrowth of Vδ1+ T cells with significant activity against neuroblastoma.[48] Knight[121] generated Vδ1+ T cells with antimyeloma activity from peripheral blood mononuclear cells (PBMNC) using a combination of PHA, IL-2, and allogeneic irradiated feeder cells. Siegers showed similar results using prolonged exposure of positively selected Concanavalin A-stimulated γδ T cells to IL-2 and IL-4 without the use of feeder cells.[115] Gamma delta T cells expanded using this protocol were still viable in a xenograft leukemia model 5 weeks postinfusion after having been injected on day 16–21 of *in vitro* culture.[122] In subsequent studies, enhanced Vδ1+ T cell expansion (up to 24,000-fold) was seen in PBMNC cultures initially stimulated with Concanavalin A, and then depleted of αβ T cells after 6–8 days.[123] Average culture duration was approximately 21 days and did not require feeders.[123] Finally, Lamb[45] was able to generate up to 1,200-fold expansion of Vδ1 T cells from PBMNC after depletion of αβ T cells and culture with irradiated leukemia feeder cells and low-level IL-2.[45]

At present, none of these protocols have direct clinical adaptability, and future methods derived thereof will require substantial modification to move forward into human trials. Such modifications should include steps to facilitate ease of handling, preferably by eliminating feeders and reducing the number of required reagents since these must be GMP/pharmaceutical grade to obtain clinical approval for therapeutic cell manufacturing.

FUTURE DIRECTIONS

It is likely that γδ T cells will have an increasing role to play in the prevention and management of malignant disease and posttransplant relapse. Our ability to harness the unique innate recognition properties of Vδ1+ T cells for therapeutic application could contribute substantially to the efficacy and duration of innate lymphocyte therapy. Initial therapeutic studies must address the distribution and function of Vδ1+ T cells following infusion, particularly with respect to the cytotoxic or regulatory phenotype and functional activity of cells that ultimately infiltrate the tumor and/or remain in the circulation.

Although not specific to Vδ1+ T cells, it has been shown in both animal models and human *in vitro* and clinical studies that

γδ T cells do not exhibit classical alloreactivity. Therefore, while γδ T cells would not be expected to recognize normal allogeneic determinants on tumor cells, they would also not pose a significant risk for initiation of graft-versus-host disease. Indeed, Drobyski[124] showed that large doses of IL-2–expanded γδ T cells could be infused into lethally irradiated MHC-disparate mice without causing graft-versus-host disease. In human studies, Schilbach[108] and Lamb[45] also found that allogeneic γδ T cells were not substantially activated in *in vitro* allogeneic mixed lymphocyte culture. Since γδ T cells can be infused with minimal risk in the allogeneic setting even after *ex vivo* activation, they offer the potential for use in settings where tumor contamination of autologous cell products may be a concern or T-cell exhaustion prevents *ex vivo* activation and expansion of autologous γδ T cells.

The recent introduction of an immunomagnetic system for depletion of αβ T cells from bone marrow or peripheral blood apheresis products will allow investigators to infuse grafts enriched for γδ T cells in lymphodepleted patients as primary grafts or donor leukocyte infusion, thereby providing a platform for homeostatic Vδ1+ T cell expansion. As noted above, however, clinical manufacturing strategies for Vδ1+ T cells have not yet matured sufficiently to permit clinical trials. The CD2/OKT3 γδ T cell expansion method described by Lopez and discussed above provides the most clinically adaptable system, as the components either currently exist in pharmaceutical grade or have been manufactured to cGMP standards in the recent past. This method would allow large numbers of Vδ1+ T cells to be manufactured, but in the absence of a specific Vδ2+ T cell depletion/Vδ1+ selection system, the product would be a composite of Vδ1+ and Vδ2+ T cells with other minor subtypes. Nevertheless, this method would produce a heterogeneous product that would incorporate the broad range of antitumor functions of each subtype over currently available methods that expand only Vγ9Vδ2 T cells. Laboratory-based methods that expand Vδ1+ T cells with greater efficiency but incorporate nonstandardized components, such as plant mitogens and/or feeder cells could potentially be moved to clinical scale. Regulatory agencies have approved trials that require feeder cells for the manufacture of cytotoxic lymphocytes when the methods could be justified by the lack of availability of similarly effective nonbiologic components and adherence to strict validation protocols.[125] As with any translation from the laboratory to the clinic, this process will likely encounter unanticipated obstacles and evolve with improvements. However, the available data strongly suggest that our ability to rapidly select and culture Vδ1+ T cells specific for a broad range of common disease- and stress-associated ligands will ensure that the advantages of this approach as part of the current therapeutic arsenal of refractory cancer therapies.

CONCLUDING REMARKS

It has been well established that γδ T cells are important mediators of cancer surveillance and could ultimately play an important role in cancer therapy. Indeed, several centers are beginning to investigate small clinical trials of Vγ9Vδ2 T cells as therapy for solid tumors. We are, however, just beginning to explore the potential therapeutic role of Vδ1+ T cells. These cells can be highly cytotoxic to epithelial and hematopoietic malignancies and have the added advantage of persistence over time, a function that has been well documented after hematopoietic stem cell transplantation. As we attain greater understanding of how Vδ1+ T cells acquire effector and immunoregulatory function, define yet-to-be described ligands for Vδ1+ T cells and appreciate the interactions of activating and inhibitory receptors with their ligands, we will be able to exploit these properties in the design of innate cell therapy strategies. Taking into consideration that the number of studies is small, it is clear nonetheless that Vδ1+ T cells play a role in the prevention of both ALL and AML relapse. The renewed interest in haploidentical stem cell transplantation and the incorporation of αβ T cell depletion into clinical graft engineering should provide opportunities to strengthen correlations between Vδ1+ T cell recovery and transplant outcomes. Vδ1+ T cells also have anti-viral properties, particularly against CMV and EBV infection, both of which have been associated with malignant transformation. How best to bring these findings into the clinic will require further study. Lastly, we urgently need to develop manufacturing strategies that will translate into the clinic if the therapeutic potential for Vδ1+ T cell-based therapies is to be realized.

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