Promising Cell-Based Immunotherapy Using Gamma Delta T Cells: Together Is Better

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Gamma delta T-cell response to cellular stress signals expressed by tumor cells makes them promising candidates for cancer immunotherapy. The proof of concept for clinical scale propagation of polyclonal $\gamma\delta$ T-cell lines with efficient in vitro and in vivo response against cancer is an important step in this direction. Clin Cancer Res; 20(22); 5573–5. © 2014 AACR.

In this issue of Clinical Cancer Research, the articles by Deniger and colleagues (1) and by Fisher and colleagues (2) report on a novel process for scaling up in vitro expansion of polyclonal $\gamma\delta$ T-cell lines with killing activity against cancer cells for clinical use. T-cell therapy to treat cancer has been the focus of much interest in the past years and led to the recent success of chimeric antigen receptors–expressing T cells for clinical use. T-cell therapy to treat cancer has been the focus of much interest in the past years and led to the recent success of chimeric antigen receptors–expressing T cells (reviewed in ref. 3). This interest mainly concentrated on $\alpha\beta$ T cells whose mode of antigen recognition, and thus of activation, has been fully resolved. Because of their ability to recognize different stress signals provided by tumor cells, $\gamma\delta$ T cells also hold an alternative promise in cancer therapy. So far, much attention has been given to the predominant subset of circulating $\gamma\delta$ T cells, the $\gamma\delta$V$\delta$2 T cells, which are strongly activated by nonpeptide phosphorylated met abolites of isoprenoid biosynthesis pathway (called phosphoantigens). Phosphoantigens are produced at high levels by tumor cells and have been successfully used to generate clinical scale quantities of V$\gamma$V$\delta$2 T cells in vitro and to expand them in vivo in active vaccination-type trials. Although remarkably safe and superior to current second-line therapies in some settings, V$\gamma$V$\delta$2 T-cell–based trials in patients with cancer provided mixed results, suggesting room for improvement in the therapeutic use of $\gamma\delta$ T cells (4).

Besides V$\gamma$V$\delta$2 T cells, all the other $\gamma\delta$ T cells (collectively called V$\delta$2$^{\text{aw}}$ $\gamma\delta$ T cells) populate many epithelial tissues where they represent an important component of intraepithelial lymphocytes, so likely the main subset of human $\gamma\delta$ T cells in the whole body, and an important first-line defense against diverse host assaults. Despite increasing evidence of their role in tumor surveillance, V$\delta$2$^{\text{aw}}$ $\gamma\delta$ T cells have been largely neglected in cancer cell therapy because of the limited knowledge about the antigens they recognize, which have restricted their specific and large-scale expansion for clinical purposes. One method to grow large quantities of whole $\gamma\delta$ T cells was developed by Lopez and colleagues (5) but has not yet been used in cell therapy trials.

Deniger and colleagues (1) and Fisher and colleagues (2) used artificial antigen-presenting cells (aAPC) for clinical scale in vitro expansion of polyclonal $\gamma\delta$ T-cell lines comprising both V$\delta$2$^{\text{aw}}$ and V$\delta$2$^{\text{aw}}$ $\gamma\delta$ T cells (Fig. 1). They took advantage of aAPCs available as clinical-grade reagents and already used to manufacture high numbers of $\alpha\beta$ T cells and NK cells for clinical trials at the MD Anderson Cancer Center (6). These aAPCs are based on K562 tumor cells genetically modified to express CD64, CD86, CD137L, and a membrane-bound form of IL15. Culturing pure $\gamma\delta$ T cells sorted from either peripheral blood mononuclear cells (PBMC) or umbilical cord blood with aAPCs, in combination with exogenous IL2 and IL21, led to a remarkably high expansion of $\gamma\delta$ T cells, providing clinical-scale amount of cells ($>10^9$ from PBMCs and $>10^{11}$ from cord blood). Although ex vivo stimulation of V$\gamma$V$\delta$2 T cells from patients with cancer is often limited using phosphoantigens, Fisher and colleagues (2) also obtained high expansion rate of $\gamma\delta$ T cells from children with neuroblastoma with this aAPC-based protocol. Using either mRNA quantification through nomenymatic digital multiplex assay (i) or next-generation sequencing (ii), both teams performed in-depth $\gamma\delta$ T-cell receptor (TCR) TCR repertoire analysis of the generated $\gamma\delta$ T-cell lines. Although not exactly reflecting the initial composition of $\gamma\delta$ T cells, the repertoire of expanded T cells was polyclonal with expression of all functional V$\delta$ and V$\gamma$ chains.

It is not yet clear which are the activation signals underpinning this large expansion of polyclonal $\gamma\delta$ T cells in this process. Proliferation was dependent on CD137L, IL2, and IL21, but the involvement of TCR signaling has not been tested. Although it is hard to conceive that aAPCs express the full array of TCR ligands for all expanded $\gamma\delta$ T cells, engagement of TCR is suggested by the low TCR expression on generated V$\delta$2$^{\text{aw}}$ T cells and by the importance of CD137L for the expansion despite the absence of CD137 expression by $\gamma\delta$ T cells prior expansion. Fisher and colleagues who coated B1 anti-$\gamma$T8CR antibody on aAPCs showed it did not have a major role in $\gamma\delta$ T-cell expansion even if this led to a better representation of V$\delta$2$^{\text{aw}}$ $\delta$ subsets. Gamma delta T cells

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have been shown to express HLA-I inhibitory receptors (7), so the absence of HLA-I expression by K562 as well as expression of activatory molecules such as NKG2D ligands may also play critical role in γδ T-cell activation.

Most interestingly, aAPC-propagated γδ T cells displayed efficient response against cancer cells, with an equivalent contribution of Vδ2iso and Vδ2iso γδ T cells. A strikingly broad killing capacity against tumor cell lines from acute or chronic leukemia, colon, pancreatic, and ovarian cancers was developed, whereas no killing of autologous or allogeneic normal B cells ensued. This panel of targets was extended to neuroblastoma cell lines in Fisher and colleagues article, which also reports on an enhanced killing in the presence of an anti-ganglioside G2 mAb consistent with the expression of CD16 on a significant proportion of aAPC-expanded Vδ2iso cells. Tumor killing was accompanied by IFNγ production, which could be an additional mean for γδ T cells to control tumors.

To test whether polyclonal γδ T cells are able to kill tumors in vivo, Deniger and colleagues (1) used an ovarian cancer xenograft model in immunodeficient mice and in vivo bioluminescence imaging. The bioburdens of established tumors were considerably reduced 72 days after injection of polyclonal or separate subsets of expanded γδ T cells, resulting in an increased long-term survival of mice.

Further investigations are needed to understand how aAPC-generated γδ T cells recognize and kill cancer cells. Combined blocking of NKG2D, DNAM1, and the TCR decreased cancer cell killing, suggesting a complex multi-molecular-based cancer cell recognition by γδ T cells. Antigen recognition by polyclonal γδ TCRs on such a broad spectrum of cancer cells raises the question of their frequency and specificity, opening perspectives for identification of new γδ TCR ligands.

These results have strong potential for translation in clinical practice. The aAPC-mediated expansion procedure and reagents have already been approved for clinical use. Autologous settings implying expansion from cells of patients with cancer are possible as shown by efficient expansion of γδ T cells from patients with neuroblastoma. Alternatively, as γδ T cells are not restricted by classical MHC and have no alloreactivity (8), infusion of γδ T cells generated from unrelated healthy donor is feasible with limited risk for GVHD, as recently done for haploidentical Vδ2iso expanded cells (9). Expression of CD16 on Vδ2iso γδ T cells also paves the way for combined therapy with therapeutic antibodies. Finally, the widely reported involvement of γδ T cells in the protection against infections might provide collateral benefit of their injection in patients with cancer susceptible to infectious diseases because of chemotherapy.

Clinical trials using aAPC-generated γδ T cells should also shed light on still pending but interesting issues: Are these cells able to migrate into the tumors? Can they expand in vivo after infusion? Is quantity of infused cells a major issue or could therapeutic potential be improved by increasing their specificity toward defined tumors or defined antigens? Altogether with the previously reported association between Vδ1 subset expansion and complete response in hematopoietic stem cell transplantation patients with leukemia (10), and the mitigated but encouraging results obtained from Vγ9Vδ2 T-cell–based cancer therapy, the infusion of polyclonal γδ T cells with broad cancer specificity deserves deep attention as a valuable additional tool in the therapeutic arsenal against cancer.

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