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Multifunctional γδ T cells and their receptors for targeted anticancer immunotherapy

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Abbreviations: allo-SCT, allogeneic stem cell transplantation; CMV, cytomegalovirus; CTE, combinatorial-γδTCR-chain exchange; GVHD, graft *versus* host disease; TCR, T-cell receptor

Human γδ T cells possess broad antitumor reactivity and are involved in the control of viral infections. We have recently described multifunctional γδ T cells induced by cytomegalovirus after allogenic stem cell transplantation, placing γδ T cells and their receptors in the spotlight for the development of novel anticancer immunotherapies.

Over the last few decades, cytomegalovirus (CMV) reactivation was considered a major life-threatening complication of allogenic stem cell transplantation (allo-SCT). Nowadays, a sensitive monitoring for early CMV reactivation combined with the availability of effective antiviral treatments has rendered the CMV-related death of transplanted patients a rare event. Fortuitously, such an improved control over CMV reactivation has facilitated observational studies in large cohorts of transplanted patients, highlighting a surprising beneficial association between CMV reactivation and a reduced risk of leukemic relapse.[1] So far, however, how viral reactivation would provide a protection from leukemic relapse has remained unclear. Among various possibilities, it has been proposed that natural killer (NK) cells may cross-react with CMV-infected cells and tumor cells by responding to CMV-infected residual AML blasts.[1] In a recent issue of *Leukemia*, we propose an additional and perhaps even more physiologically relevant explanation for this apparent paradox, i.e., that γδ T cells play a pivotal role in the CMV-induced clearance of residual tumor cells.[2] We observed that these unconventional T cells not only expand in patients that reactivate CMV upon allo-SCT but also react against both CMV-infected and leukemic cells. Thus, we propose that multifunctional γδ T cells could substantially contribute to the protection from leukemic relapse that is associated with CMV reactivation after allo-SCT.

In humans, circulating γδ T cells are a minor population that mostly expresses T-cell receptors (TCRs) containing the Vδ2 and Vγ9 gene segments (so-called $V\delta2^{pos}$ γδ T cells).[3] In contrast, epithelial γδ T cells mainly express TCRs composed of Vδ1 or Vδ3 chains ($V\delta2^{neg}$ γδ T cells) optionally in combination with CD8αα. Over the last decade, many studies have implicated $V\delta2^{neg}$ γδ T cells in the anti-CMV response as well as in antitumor immunosurveillance, but the first report on the cross-reactivity of these cells against CMV and cancer originated from the isolation of $V\delta2^{neg}$ γδ T-cell clones from kidney transplant recipients.[4] At least in some of these clones, the double reactivity was mediated by a γδ TCR recognizing a common stress antigen that was upregulated on both CMV-infected and transformed intestinal epithelial cells,[5] explaining why CMV infection alone could induce an immune cell population reacting against both CMV and cancer. Conversely, in our study, gene transfer experiments using γδ TCRs isolated from cross-reactive $V\delta2^{neg}$ γδ T-cell clones demonstrated the crucial involvement of the γδ TCR in tumor reactivity but not in the recognition of CMV-infected cells, suggesting that—at least in these $V\delta2^{neg}$ γδ T-cell clones—anti-CMV reactivity relied on receptors other than the γδ TCR. This brings up a major issue in the γδ T-cell research field, that is, the poor understanding of the mechanisms of γδ T-cell activation as well of the antigens recognized by γδ TCRs. In this respect, one important finding of our study is the identification of CD8αα as a co-stimulatory molecule for the activation of defined γδ TCRs. The expression of CD8αα on γδ T cells has previously been described, yet so far there were no reports on its function. In αβ T cells, the CD8αβ heterodimer serves as co-receptor for the αβ TCR, restricting its interaction to antigens presented on MHC Class I molecules. Conversely, γδ TCRs recognize antigens independently of MHC molecules, suggesting that the co-activating function of CD8αα is likely to rely on alternative mechanisms. The precise mechanisms whereby CD8αα delivers co-stimulatory signals in this setting remain to be elucidated. Nevertheless, we observed a striking increase of circulating $CD8\alpha\alpha^{+}$ γδ T cells in CMV-reactivating individuals in our patient cohort as well as in an additional independent cohort

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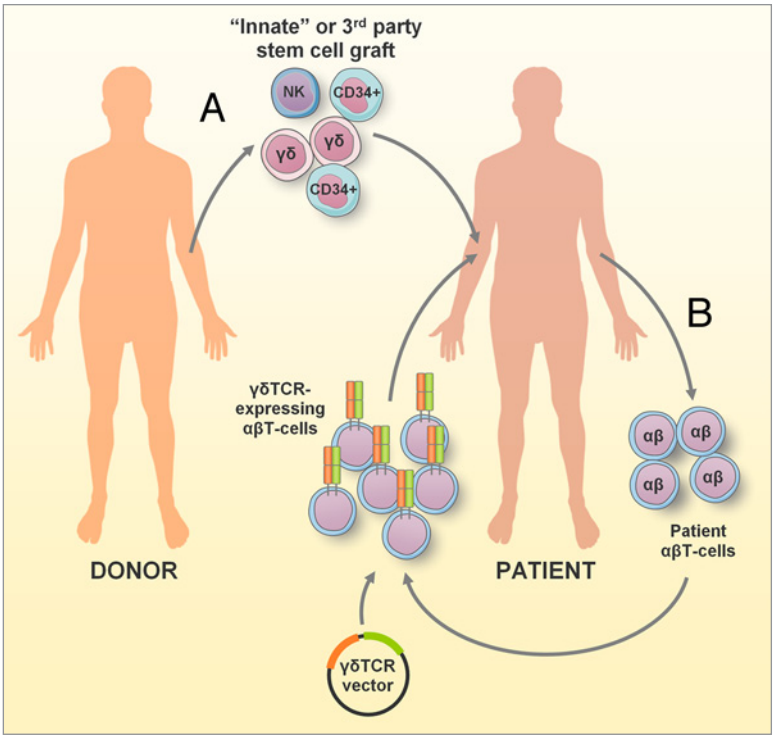

Figure 1. Antitumor strategies based on γδ T cells. (**A**) The cell preparation for an "innate allogenic stem cell transplantation" (allo-SCT) from conventional or third party donors may selectively contain or be enriched for γδ T cells to provide anti-cytomegalovirus (CMV) and antitumor protection in the absence of graft versus host disease (GVHD). (**B**) In a complementary "autologous engineered transplantation," T cells are isolated from cancer patients, expanded and engineered to express γδ TCRs (optimized by combinatorial-γδTCR-chain exchange) ex vivo. Reprogrammed T cells are subsequently re-infused into the patient, where they specifically recognize and kill tumor cells.

of congenitally CMV-infected neonates, implying that CD8αα expression by γδ T cells represents a pathophysiologically relevant phenomenon in vivo.

The demonstration that a proportion of γδ T cells can cross-recognize CMV and a broad panel of hematological cancer cells make them particularly attractive for clinical applications, such as adoptive cell transfer-based immunotherapy. In the context of allo-SCT, a situation favoring the reactivity of re-infused cells against CMV and leukemia in the absence of graft vs. host disease (GVHD) might be achieved with stem cell grafts enriched for γδ T cells (**Fig. 1**). To this end, we and others are nowadays conducting clinical trials using stem cell grafts depleted of αβ T cells and B cells (NTR2463 and NTR3079).[6] Intriguingly, αβ TCR/CD19-depleted, but usually not CD3/CD19-depleted, grafts reconstitute very rapidly a broad αβ T-cell repertoire (J Kuball, unpublished observations), suggesting a very broad immunoregulatory role for γδ T cells that has recently also been proposed by others.[7] As an alternative, umbilical cord blood grafts can be used as a third party source of stem cells. These grafts typically contain high percentages of γδ T cells, and we have demonstrated that CMV- and leukemia-reactive γδ T cells can also be obtained from such an antigen-naïve repertoire. Importantly, all good manufacturing practice-grade clinical tools for the preparation of enriched stem cells grafts are available.

Finally, as our results suggest a central role for CD8αα$^+$ γδ T cells in anti-CMV immune responses, the isolation of these cells could be envisioned, although their precise function would have first to be deeply investigated.

Complementary to this "innate allo-SCT" approach, γδ TCRs with broad tumor-reactivity could be characterized and used to reprogram patient-derived conventional αβ T cells[8] (**Fig. 1**). Given the non-MHC-restricted antigen recognition pattern of γδ TCRs, defined γδ TCRs could—in contrast to αβ TCRs—be applied to a broad patient population in the absence of matched HLA types. Also, exogenous γδ TCR chains do not pair with their endogenous αβ counterparts, preventing the creation of novel TCRs with unpredictable (auto-) reactivity. As we have previously shown, introducing defined γδ TCRs effectively reprograms αβ T cells to kill a broad collection of tumor cells in vitro and in vivo.[9] In this setting, we also established a technique called combinatorial-γδTCR-chain exchange (CTE), allowing for the design of γδ TCRs with enhanced functional avidity toward malignant but not healthy tissues.[10] By exploiting the abundance, potent cytotoxic machinery and proliferative competence of αβ T cells even in advanced stages of disease, the engineering of autologous immune cells with such receptors would allow for the generation of large numbers of tumor-reactive T cells while tackling the major limitations of current approaches based on engineered αβ TCRs. Thus, γδ T cells and their receptors stand out as a promising avenue toward the development of new antitumor immunotherapies.

Disclosure of Potential Conflicts of Interest

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