Engineering effective T-cell based antitumor immunity

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The adoptive transfer of synthetic siRNA-mediated cblb-depleted autologous CD8+ T cells acts as a potent adjuvant for dendritic cell (DC) vaccination and provides a significant therapeutic benefit. Our proof-of-concept study validates the strategy of inhibiting CBLB as a rational approach to augment the effectiveness of adoptively transferred immune cells.

The concept to harness a patient’s immune system against cancer is an emerging strategy. The adoptive transfer of autologous T cells to enforce tumor-cell killing by immune mechanisms has indeed shown promising results in the treatment of various types of cancer, especially malignant melanoma. A major drawback of current clinical applications of adoptive cell transfer (ACT), however, is that they generally require laborious ex vivo expansion and/or genetic engineering procedures to generate a potent tumor-reactive CD8+ T-cell phenotype. These interventions bear the risk of insertional mutagenesis, e.g. as a result of the inappropriate insertion of T-cell receptor-coding lentiviral vectors within proto-oncogenes, potentially causing T-cell leukemogenesis. Moreover, the therapeutic efficacy of ACT appears to be limited by immune evasion mechanisms that are in place in the tumor-bearing host, such as those mediated by the secretion of transforming growth factor β (TGFβ) in the tumor microenvironment and by the accumulation of regulatory T cells, both of which severely dampen the in vivo activation, expansion, and tumor homing of transferred tumor-reactive CD8+ T cells. Another obstacle on the way to broadly applicable ACT therapies is the restricted knowledge of tumor antigens that are capable of eliciting potent anti-tumor T cell responses. It is therefore desirable to establish strategies that enhance the effector functions of adoptively transferred CD8+ T cells in vivo but minimize the ex vivo manipulation of these cells prior to transfer. We have recently demonstrated that an ex vivo synthetic small-interfering RNA (siRNA)-based approach that depletes casitas B-lineage lymphoma proto-oncogene b (CBLB) constitutes a rational strategy to achieve such goals, as it profoundly improves the efficacy of ACT for cancer immunotherapy.1

CBLB

CBLB is a member of the mammalian family of RING E3 ubiquitin ligases and has been shown to mediate the CD28 dependence of T-cell activation.2–3 Accordingly, the loss of cblb leads to anergy resistance and susceptibility to autoimmunity. CBLB also contributes to the maintenance of self-tolerance by mediating the immunosuppressive effects of TGFβ. T cells from cblb knockout mice are less sensitive to TGFβ stimulation in vitro and in vivo,4 and are resistant to inhibition by regulatory T cells, which constitute a major source of TGFβ.5 Moreover, our group and others demonstrated that cblb-/- mice can reject tumors in a CD8+ T cell-dependent fashion, a process that could be attributed—at least in part—to the hyporesponsiveness to TGFβ of cblb-deficient T cells.6–9

Proof of Concept 1: siRNA-mediated cblb Silencing Prior to ACT Provides Significant Therapeutic Benefits

Cell transfer studies with knockout T cells have previously suggested that inactivation of CBLB could represent a rational approach to improve anticancer T-cell reactivity in vivo. As a proof of concept study in support of the clinical development of this strategy, we established a synthetic cblb-targeting siRNA transfection protocol on polyclonal CD8+ T cells to be used for ACT, aiming at increasing the efficacy of a dendritic cell (DC)-based vaccine. cblb-silenced CD8+ T cells were hyperresponsive and mostly protected from the suppressive effects of TGFβ in vitro. This translated into a markedly increased reactivity against a subcutaneously injected ovalbumin-expressing B16 tumor, as reflected by enhanced tumor infiltration and interferon (IFN)γ secretion by transferred cblb-depleted CD8+ T cells. As a consequence, the combination of a DC-based vaccine with the adoptive transfer of cblb-silenced CD8+
T cells resulted in a strong suppression of tumor growth and substantially prolonged overall survival. Of note, such antitumor effects were observed without any signs of autoimmunity. This suggests that the autimmune side effects of the therapy will not hamper a potential clinical translation of the concept.

In our study, we used as a model antigen for vaccination not only the ovalbumin-derived SIINFEKL peptide, but also the established melanoma antigen gp100. Remarkably, however, adoptively transferred CD8+ T cells were polyclonal, i.e., they were not carrying a transgenic T-cell receptor specific for any of the antigens targeted by the vaccine. It is also noteworthy that in our system ACT was not combined with lymphopenia, as it frequently is in a variety of preclinical and clinical settings.

Using polyclonal T cells for ACT would greatly simplify the standard operating procedure in the clinic. Furthermore, as tumor-specific antigens that underpin tumor eradication are often not known, using hyperreactive polyclonal T cells is a feasible approach. Last but not least, a major benefit of transferring polyclonal T cells is their broader reactivity against a variety of tumor antigens. In this setting, it should be more difficult for neoepitope-presenting cells to escape the immune attack by simply downregulating a specific tumor antigen.

**Proof of Concept 2:**

The Consequences of cblb Silencing in Human T Cells are in Agreement with Those Observed in Murine T Cells

To transfer this approach into the human setting, we established a similar procedure for ex vivo depletion of cblb in human CD8+ T cells. In agreement with the phenotype observed in murine T cells, the knockdown of cblb in human CD8+ T cells significantly enhanced IFNγ production, even in the absence of CD28 co-stimulation. Moreover, the cblb mRNA was almost undetectable even one week after transfection. This suggests that, in a therapeutic setting, antitumor CD8+ T cells should be hyperreactive for at least several days.

**Conclusion**

Taken together, our findings have laid the groundwork for an adjuvant cancer immunotherapy project pursued by the Medical University of Innsbruck and Apeiron Biologics (http://www.apeiron-biologics.com/) that aims at optimizing existing therapeutic regimens and at identifying potential additive or synergistic effects to obtain long-term clinical responses against cancer (Fig. 1).

**Disclosure of Potential Conflicts of Interest**

No potential conflicts of interest were disclosed.

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