Improving the Anti-Cancer Effect Of T-Cells by T-Cell Receptor Engineering

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There are two populations of T-cells that are characterized by the type of T-cell receptor (TCR) that is expressed: αβ and γδ. The majority of T-cells express αβ TCRs while only 2% of total T-cells have TCRs made of γδ subunits. Furthermore, a specific type of γδ T-cell that expresses γδ2TCRs, a class of innate immune cells, can be highly anti-tumorgenic in mouse cancer models. However, adoptive transfer of γδ2 cells in the clinic has provided less tumor control. The Strong (Basic Sciences Division) and Kuball (UMC Utrecht, The Netherlands) labs investigated two important questions: what is the molecular defect curtailing the anti-tumor effectiveness of γδ2T-cell therapy and can this weakness be ameliorated to increase their therapeutic potential?

As a starting point, γδ2T-cells from a healthy donor were cloned and their reactivity against a panel of tumor cells was assessed by an IFNγ ELISA assay. Individual clones of γδ2T-cells displayed differential recognition of and activation by specific tumor cells. They hypothesized that the strength of the anti-tumor response correlated with differences in γδ2TCR amino-acid sequence. To test this, the γδ chains of a T-cell clone with strong functional avidity was sequenced and compared to a T-cell clone that displayed a weak response in the ELISA assay. Interestingly, only the CDR3 domain of the receptors contained amino acid changes. To determine if the amino acid changes influenced TCR function, the mutant receptors that displayed increased functionality and wild-type control receptors were transduced into peripheral blood αβT-cells and T-cell activity against tumor cells was determined. Indeed, αβT-cells expressing the stronger γδ2TCR had a greater ability to lyse tumor cells and secrete IFNγ compared to wild-type γδ2TCR-expressing αβT-cells. Thus, the CDR3 domain of the γδ2TCR is a critical determinant of TCR functionality.

Mutational analysis of CDR3 domain residues in both the γ9 and δ2 chains of wild-type receptors revealed that too short (no amino acid addition) and very long (12 amino acid) alanine stretches in a particular CDR3 region negatively affect receptor function. To determine if an optimal CDR3 length exists, they searched the ImMunoGeneTics database for stretches of amino acids in the CDR3 regions of the γδ2TCRs. The majority of listed γδ2TCRs contained a conservative number of residues (5-7 amino acids) in the CDR3 domain, indicating that those TCRs have a functional advantage and are under positive selection. In addition, single point mutations in both chains of the
γ9δ2TCRs were found to influence TCR functional avidity. Lastly, CTE (combinatorial-γδTCR-chain-exchange) was used to engineer γ9δ2TCRs that were predicted to have increased receptor function based on the aforementioned results. Astoundingly, αβT-cells expressing the CTE-engineered γ9δ2TCRs were highly reactive against a broad range of tumors and did not affect normal tissue in the IFNγ ELISA. When tested in a humanized mouse model, the CTE-engineered γ9δTCRs decreased tumor outgrowth and increased the overall survival of the mice. Taken together, these data indicate that CTE-engineered TCRs are a promising candidate for clinical application and may prove useful for future anti-cancer therapy.


A scanning electron micrograph of a T lymphocyte (Tc) recognizing and attaching to an antigen presenting cell (APC).

Obtained from Institut Pasteur website.