1133 CHARACTERIZATION OF CD4+ T CELL RESPONSES AGAINST RHO C, A METASTASIS ASSOCIATED TUMOR ANTIGEN

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Background CD4+ T cells are conventionally thought of as mainly 'help providers' in anti-cancer immune responses, supporting the generation of robust CD8+ T cell responses. However, more recently CD4+ T cells and their role in anti-cancer responses have gained new interest based on data suggesting that CD4+ T cells are often induced in high frequency in therapeutic vaccination against cancer. Recently, vaccination with a RhoC derived long-synthetic peptide (RV001) induced a long-lasting CD4+ T cell response in prostate cancer patients.[1] Nonetheless, it is not known whether endogenous RhoC protein is processed and presented as peptides in the context of HLA Class II molecules for recognition by CD4+ T cells. Similarly, it remains unclear whether the CD4+ T cells induced by the vaccine have the ability to directly kill cancer cells. To address these questions, we mimicked vaccine-induced RV001 specific CD4+ T cells *in vitro* and tested their capacity to kill cancer cells expressing their cognate antigen.

Methods T cells were isolated from the peripheral blood of a healthy donor and stimulated with peptide-pulsed autologous dendritic cells or PBMCs for five rounds. After each stimulation round, peptide reactivity was evaluated using EliSpot and intracellular cytokine staining (ICS). Peptide specific CD4+ T cells were sorted based on cytokine secretion and subsequently cloned by limiting dilution. Expanded clones were tested for cytotoxicity against FM3 cancer cells using xCELLigence Real-Time Cell Analysis and T cell receptor (TCR) assessment was performed using flow cytometry and polymerase chain reaction (PCR).

Results After multiple rounds of stimulation, we successfully generated RV001-specific CD4+ T cells from a healthy donor. These reactive CD4+ T cells exhibited production of TNFα and IFNγ, along with the surface expression of the degranulation marker CD107a. Upon restimulation with the peptide, the generated CD4+ T cell clones demonstrated robust production of TNFα and IFNγ, as well as the synthesis of the cytotoxic molecules granzyme B and granulysin. Next, all clones showed cytotoxic activity upon peptide-pulsed FM3 cancer cells. For two of the clones, killing of unpulsed FM3, which express endogenous RhoC, was seen. Notably, the same TCR beta variable chain was found across the generated clones.

Conclusions We showed that peptide-specific CD4+ T cells can produce cytotoxic molecules and mediate tumor cell killing. These findings offer a glimpse of the possible benefits of targeting CD4+ T responses in therapeutic cancer vaccination.

REFERENCE

1. Schuhmacher J, Heidu S, Balchen T. Vaccination against RhoC induces long-lasting immune responses in patients with prostate cancer: results from a phase I/II clinical trial. *J Immunother Cancer*. 2020;**8**:e001157

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