Identifying the Targets of Perforin Mediated Killing

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Upon activation CD8+ effector T cells develop cytotoxic function, suppress antigen presentation and undertake pathogen clearance via perforin mediated killing. However the targets of the perforin mediate killing remains unknown. In this study we developed a chimeric protein called Sensor of Perforin Ordained Targets (SPOT) to identify the targets of prf mediated killing. The SPOT construct contains a mutant Prf which is expressed at WT levels but is nonfunctional. Excellent antibodies exist for human perforin, making flow cytometric detection of surface bound Prf feasible. However, the amount of perforin secreted onto the target cells in physiological context would likely be limiting for efficient detection by flow cytometry. Therefore, the mutant prf is attached to a biotyinylating moiety-Turbo ID via a long linker. Adding Turbo-ID molecule, a bacterial biotinylase protein used for probing protein: protein interaction, would help biotinylate the surface proteins on target cells allowing for amplified detection via flow cytometry. This protein would be packaged inside cytotoxic granules of Prf deficient T cells and released appropriately and adhere to the target cells without disturbing the membrane integrity or delivering the Granzyme molecules into the cytoplasm. Using Site Directed Mutagenesis, we were able to demonstrate that a gain of glycosylation mutation in Prf renders the protein non-functional without interfering with its expression. The transgenic T cells, transduced with the mutant prf demonstrated a remarkable loss of cytotoxic function as observed via 7AAD staining, when cultured with target cells pulsed with cognate antigen. Therefore, we hypothesize that an optimized chimeric SPOT protein transgenically expressed in T cells or NK cells will reveal the normal *in vivo* targets of perforin-mediated killing after infection and elucidate the mechanisms of perforin-mediated immune regulation in unprecedented detail.