Discovery of a novel perforin inhibitor within cytotoxic granules that halts RBC lysis and reduces T lymphocyte killing.

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A primary mechanism by which T cells clear targets is by the cytotoxic granule pathway. Perforin protein (Pfn) is an essential component of the granule mediated pathway, permeabilizing the target cell's plasma membrane to apoptotic granule proteins and contributing to osmotic lysis. Here we describe an inhibitor of perforin within the granules themselves that is capable of ablating red blood cell (RBC) lysis by purified cytotoxic granules or by recombinant perforin, and that blocks allogeneic killing by T lymphocytes. The source of the inhibitor was the dense granules of murine $Pfn^{-/-}$ T lymphocytes that were expanded under high IL-2 activating conditions, purified using Percoll gradients, and extracted. The perforin inhibitory activity was limited to the dense $Pf^{-/-}$ granule fraction where cytotoxic proteins reside. The ability of wild type granules to lyse RBCs was reduced several fold by $Pfn^{-/-}$ KO granules at less than 3 ug/ml total granule protein. Recombinant perforin, free of any other granular cofactors, was similarly reduced in lytic capability by $Pfn^{-/-}$ granules. When $Pf^{-/-}$ granules were applied to allogeneic T lymphocyte reactions against P815 target cells, the effector cells demonstrated up to 2 fold reduction in effectiveness. Unlike perforin, the inhibitor is not calcium sensitive, nor does the inhibitor extensively degrade perforin protein as assayed by immunoblot. It is heat-inactivated, which suggests that the activity is independent of sulfated glycans. It is also unaffected by E64, a cysteine protease inhibitor that affects cathepsin B, a granule enzyme that P. Henkart *et al.* have implicated in the regulation of perforin lysis (Ref). Our working hypothesis is that the inhibitory factor contributes to intricate mechanisms that regulate perforin-mediated cytotoxic processes.