Mass Spectrometric Proteomic Analyses of Proteins from Murine Cytotoxic T Cell Granules.

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Perforin from cytotoxic lymphocytes provides a portal for several deadly granzymes and granulysin to enter cells and activate apoptotic mechanisms. We hypothesized that additional, unidentified granule proteins could promote death if they gained entry into cells and undertook a proteomics approach to identify new granule proteins. Cytolytic granules were isolated from two different mouse T cell lines using Percoll gradients. Protein extracts were separated by 2D (isoelectric focusing and SDS-PAGE) gel electrophoresis, the proteins stained with SYPRO Ruby, 'spots' of protein digested with trypsin and the mass spectra determined for the trypsin fragments and for further ionized fragments of selected peptides from each protein. There were 250-500 protein spots per 'granule' extract. Identification of the 'tryptic fingerprints' indicated that many of these proteins were contaminants. Five proteins were identified as granule or granule-associated (perforin, granzymes A, B and C, and gamma-actin). They represented a small fraction of the total SYPRO Ruby intensity. Technical obstacles included inconsistent mobility of specific proteins and multiple mobilities (spots) for a single in the 2D gels. With refinement of the techniques, it may be possible to characterize post-translational modifications of the 19 known proteins in cytotoxic granules. It may be necessary to examine exocytosed proteins to reduce the number of false-positive proteins and to distinguish genuine granule proteins. Supported by NIH R01CA38942.