Insights into perforin-assisted granzyme delivery

Cytotoxic lymphocytes kill infected, tumor and auto-reactive cells by secreting the content of the cytotoxic granules into the immunological synapse. The primary components of cytotoxic granules are perforin (PFN) and granzymes, a family of pro-apoptotic serine proteases. Currently, two models exist for the delivery by PFN of granzymes into target cells to induce apoptosis. The first involves formation of plasma membrane pores by perforin oligomers which allow passage of granzymes directly into the cytosol, while the second model proposes that the delivery of the granzymes occurs by an endosomolytic process in which PFN may play a role in releasing granzyme from the endosome. We studied the effects of PFN on lipid bilayers and size of the pores it forms with different approaches such as giant unilamellar vesicles (GUVs), planar lipid bilayers (PLM) and surface plasmon resonance (SPR). We showed on GUVs and PLM that PFN pores exist with a range of functional diameters. Moreover, our results indicate that the PFN pores allow the passage of fluorescent dextrans with MW 10 kDa or smaller. Fluorescently-labeled granzyme B was also able to cross the membrane in such conditions. However, the passage of GrB is process through the pore appears to be rather slow process. In addition to pore formation, we have also observed that PFN can induce the formation of invaginations on GUVs. By using the fluorescently labeled antibodies we showed that the PFN is not uniformly distributed over the whole GUV surface but rather concentrated in the invaginations. The process of invagination starts immediately after PFN addition and leads to formation of secondary vesicles in GUVs, which are filled with the surrounding medium. We hypothesize that the invaginations induced by PFN may be physiologically relevant for GrB delivery in vivo.