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Methodology to Understand How Perforin Assembles on Membranes

To the Editor:

Cytotoxic T lymphocytes (CTL) and natural killer (NK) cells form a part of the immune system and play crucial roles in defence against virally infected and neoplastic cells. They are involved in the elimination of the target cells predominantly through perforin/granzyme apoptosis pathway. Upon the formation of the immunological synapse (IS) between a CTL/NK and target cell, CTL/NK cytotoxic secretory granules are fused to the CTL/NK cell membrane and release contents into the IS. Transmembrane pore-forming protein perforin (PFN) and granzyme (Grz) serine protease are critical effector molecules in CTL/NK cytotoxic granules [1]. After releasing of components into the IS, PFN monomers bind to the target cell membrane and facilitate granzymes delivery to the target cell cytosol. Consequently, granzymes attack to different protein substrates, led to activation of the apoptotic cascade enzymes and initiated programmed cell death [2].

Although several decades have passed from discovery of PFN and granzymes, the exact mechanism of granzyme trafficking across membrane bilayer is remained enigmatic. However, to date, several remarkable models have suggested to the function of PFN. Based on the structural homology between PFN and complement components, it was proposed that multimerization of PFN on target cell membrane led to formation of stable transmembrane pores and granzymes are delivered directly into the cytosol. This classic model was questioned when it was demonstrated that granzymes can enter the target cells independently of PFN, presumably through direct interaction with the cell surface receptor. It is interesting that the model was modified by Froelich *et al.*, who proposed that the passage of the granzymes occurs by an endosomolytic process. According to the presented model, PFN does not act on the cell membrane, but rather at the endosomal membrane, to release granzymes from endosomes into the cytosol, possibly via pore formation on the endosomal membrane. In spite of the revised model by Froelich *et al.*, it has not been widely accepted. Hence, Lieberman and Pipkin have suggested a hybrid model, in which they suggest that for granzymes delivery, PFN acts on both cell and endosomal membranes [3]. Despite of the suggested models, the precise molecular mechanism of pore formation by PFN on plasma or endosomal membrane is not fully understood [4].

Perforin is a member of the MACPF superfamily of pore-forming proteins and consists of 533 amino acid and three domains: N-terminal membrane attack complex/perforin (MACPF), epidermal growth factor like domain and a C-terminal calcium-binding C2 domain [2]. Perforin monomers bind to the target cell membrane phospholipids in a Ca^{2+}-dependent manner, and the C2 domain is needed for this process. Previous investigation has suggested that the MACPF domain is responsible for membrane insertion and pore formation [5]. The conformational change in PFN monomers led to the polymerization and assembly of them in pores with a diameter of approximately 20 nm, which are suitable to transmission of the pro-apoptotic granzymes into the target cell cytosol [4].

As mentioned above, the precise molecular mechanism of pore formation by PFN on plasma or endosomal membranes is not fully understood. Here, we would like to introduce an approach that may be clarified molecular and submolecular details of this mechanism. There are many questions in regarding to the pore formation mechanism of PFN, which is may be answered with molecular dynamic (MD) simulation techniques. In the past years, these techniques are widely applied to study of the biological processes that are not directly accessible via experiment.

Importantly, structural studies have demonstrated that due to similarity between the structures of the MACPF domain and the pore-forming domain of the cholesterol-dependent cytolysin (CDC) protein from Gram-positive bacteria, both protein families use a similar mechanism for transmembrane pore formation [6, 7]. Recently, Ohkura *et al.*, investigated the membrane-binding mechanism of *Intermedilysin*, a human specific pore-forming cytolysin, using the MD simulation method [8]. In addition, several studies have used this procedure to disclose the mechanisms of pore formation [9, 10]. Therefore, it seems that the MD simulation approach could be applied as a high enough sensitivity and beneficial approach to elucidate the details of the pore formation mechanism by PFN in the membrane of target cells.

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