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Cytolytic granules supply Ca^{2+} for their own exocytosis via NAADP and resident two-pore channels

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When cytotoxic T-lymphocytes (CTLs) kill infected or cancerous cells they secrete cytolytic proteins (perforin and granzymes) into the target cell. These "death factors" are pre-stored in cytolytic granules within the CTL until an increase in the intracellular Ca^{2+} drives granule exocytosis. However, not all sources of Ca^{2+} stimulate exocytosis: we have recently demonstrated that it is the cytolytic granules themselves that are the source of the Ca^{2+} that most efficiently drives their own exocytosis; release of Ca^{2+} from the granules is only activated by the Ca^{2+}-mobilizing messenger NAADP (nicotinic acid adenine dinucleotide phosphate) that acts upon target two-pore channels (TPCs) present on the granules. That NAADP is a unique stimulus of exocytosis may be of fundamental importance not only to immunology but to cell biology in general.

Keywords: NAADP, calcium, exocytosis, T-cell, TPC

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The role of acidic Ca^{2+} stores in immune cells is, at present, poorly understood but our recent findings indicate that they may be crucially important for fighting infection. One powerful mechanism that cytotoxic T lymphocytes (CTLs) use to kill virus-infected or tumorigenic target cells is the exocytosis of cytolytic proteins (e.g., granzymes and perforin) at the immunological synapse formed at the contact interface between the CTL and its target cell.[1,2] These "death factors" are pre-stored in specialized secretory lysosomes (termed cytolytic granules) within the CTL until an increase in intracellular Ca^{2+} concentration drives granule exocytosis.[3]

A trigger for CTL stimulation is the activation of its T-cell receptor (TCR) by antigen-MHC complexes on the target cell resulting in a biphasic elevation of Ca^{2+} i.e., an initial release of Ca^{2+} from intracellular stores followed by Ca^{2+} entry across the plasma membrane. Because intracellular Ca^{2+} release in T-cells can make a relatively small contribution to the global Ca^{2+} signal, Ca^{2+} influx has understandably garnered more attention and the store-operated Ca^{2+} influx pathway [Stim/Orai, that is regulated by the Ca^{2+}-filling state of the endoplasmic reticulum, (ER)] has been implicated in supporting granule exocytosis and target-cell killing.

Nonetheless, Ca^{2+} release from intracellular stores may assume a greater importance than merely "tuning" Ca^{2+} influx. A theme in Ca^{2+} signaling is that not all sources or patterns of intracellular Ca^{2+} are equivalent and Ca^{2+} channels can differentially couple to cellular processes.[1,4] In CTLs, the exocytosis of cytolytic factors is clearly Ca^{2+}-dependent; however, although store-operated Ca^{2+} influx across the plasma membrane is a necessary component for the exocytosis of cytolytic proteins, it is not a sufficient stimulus per se because it requires the additional activation of protein kinases.[5,6] We therefore hypothesized that a different Ca^{2+} channel family couples more directly to exocytosis.

Although the ER is the largest and the best-characterized Ca^{2+} store, acidic organelles (e.g., endo-lysosomes) are emerging as important Ca^{2+} stores but ones using a different second messenger pathway to the familiar Ins(1,4,5)P_3: acidic Ca^{2+} stores are preferentially mobilized by the Ca^{2+}-mobilizing messenger,

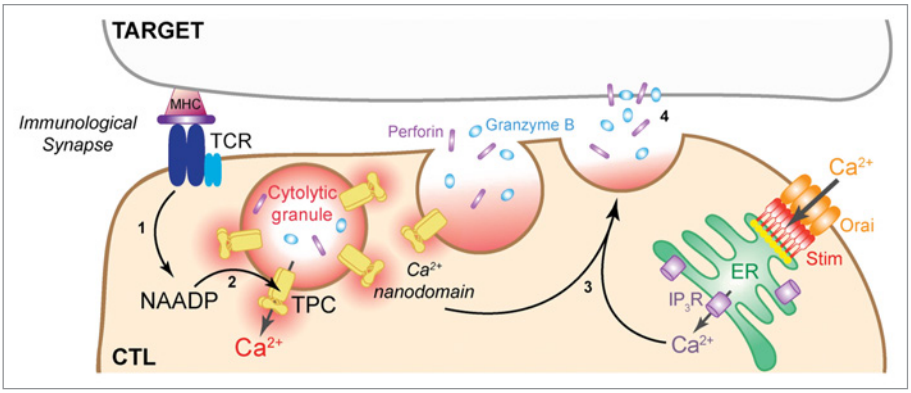

Figure 1. Activation of the T-cell receptor (TCR) by contact with the target cell results in Ca^{2+} signals in the CTL: (1) the second messenger, NAADP, is synthesized; (2) NAADP activates target TPCs (two-pore channels) on the acidic cytolytic granules themselves; (3) TPCs generate local Ca^{2+} domains around the granules that act in concert with the ER/Ca^{2+} influx pathway to evoke exocytosis (4).

nicotinic acid adenine dinucleotide phosphate (NAADP) that activates two-pore channels (TPCs).[7-10] We tested whether this pathway is important for granule exocytosis and cell killing in CTLs.[11]

Using pharmacological and genetic approaches, we first showed that this acidic Ca^{2+} store/NAADP/TPC pathway was present and contributed to TCR-stimulated Ca^{2+} signals.[11] More remarkably, NAADP-induced Ca^{2+} release could drive exocytosis of cytolytic granules whereas the Ins(1,4,5)P_3/Orai system or ionomycin (Ca^{2+} ionophore) were ineffective i.e., there was a selectivity for the acidic Ca^{2+} store pathway and NAADP.[11] A further twist in the tale was that the targets for NAADP, TPCs, were on the cytolytic granules themselves, suggesting that the granules served a dual function: they contributed to as well as responded to Ca^{2+} signals to efficiently drive their own exocytosis.[11] We hypothesize that privileged *local* Ca^{2+} nanodomains around TPCs on the acidic Ca^{2+} stores are sensed by the neighboring exocytotic machinery and this locally high Ca^{2+} is what distinguishes NAADP from the other stimuli.

In summary, TCR activation recruits NAADP to activate target TPC channels resident on the exocytotic granules themselves, and TPCs consequently translocate toward the immunological synapse. Therefore, these granules store and release the Ca^{2+} for their own exocytosis and deliver Ca^{2+} in an "autocrine" fashion via TPCs, presumably acting in local perigranular Ca^{2+} nanodomains (**Fig. 1**). That NAADP is a unique stimulus of exocytosis may be of fundamental importance not only to immune cell function but may impact on stimulus-secretion coupling in wider cellular contexts.

Disclosure of Potential Conflicts of Interest

No potential conflicts of interest were disclosed.

Acknowledgments

Figure 1 is reprinted from the graphical abstract from Current Biology 22, 2331–2337, Davis L. C., et al. NAADP activates two-pore channels on T cell cytolytic granules to stimulate exocytosis and killing, Copyright (2012), with permission from Elsevier.

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