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Caspase-1 Activation of Lipid Metabolic Pathways in Response to Bacterial Pore-Forming Toxins Promotes Cell Survival

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SUMMARY

Many pathogenic organisms produce pore-forming toxins as virulence factors. Target cells however mount a response to such membrane damage. Here we show that toxin-induced membrane permeabilization leads to a decrease in cytoplasmic potassium, which promotes the formation of a multiprotein oligomeric innate immune complex, called the inflammasome, and the activation of caspase-1. Further, we find that when rendered proteolytic in this context caspase-1 induces the activation of the central regulators of membrane biogenesis, the Sterol Regulatory Element Binding Proteins (SREBPs), which in turn promote cell survival upon toxin challenge possibly by facilitating membrane repair. This study highlights that, in addition to its well-established role in triggering inflammation via the processing of the precursor forms of interleukins, caspase-1 has a broader role, in particular linking the intracellular ion composition to lipid metabolic pathways, membrane biogenesis, and survival.

INTRODUCTION

Pathogenic micro-organisms, and in particular bacteria, often produce pore-forming proteins as virulence factors (van der Goot, 2001). These proteins are either bonafide pore-forming toxins or components of type III secretion apparatus involved in perforating the host plasma membranes to allow the injection of bacterial effectors necessary for infection (Mota et al., 2005). The size of the pores varies from 1–2 nm for pore-forming toxins such as aerolysin from *Aeromonas hydrophila* (Abrami et al., 2000) or α-toxin from *Staphylococcus aureus* (Bhakdi and Tranum-Jensen, 1991), to 25–30 nm for Cholesterol Dependent Toxin such as pneumolysin or listeriolysin O (Shatursky et al., 1999). Depending on the toxin, its concentration, and the type of target cell, the outcome of the toxin-induced plasma membrane perforation may also vary. The two extreme cases are osmotic lysis of erythrocytes, which are not necessarily physiological targets of these toxins, and full-membrane repair and cell survival (Walev et al., 2001; Walev et al., 1994). The most common outcome in vitro is however cell death—which can be apoptotic or necrotic but has generally not been characterized (Abrami et al., 2000). Little is known however about the underlying cellular mechanisms.

Here we were interested in cellular responses to pore-forming toxins, in particular survival mechanisms. We used aerolysin as a prototype since it is well characterized (Abrami et al., 2000), and a variety of inactive mutants are available (Fivaz et al., 2002; Tsitrin et al., 2002). The toxin is secreted by *Aeromonas* species as an inactive precursor, called proaerolysin, which must be proteolytically processed by gut enzymes or by proteases of the furin family (Abrami et al., 2000). The precursor, as well as the mature toxin, binds specifically to GPI-anchored proteins at the surface of target cells (Abrami et al., 2000). Once bound and processed, the toxin heptamerizes into a circular ring, a process that is promoted by lipid rafts (Abrami and van der Goot, 1999). Toxin oligomerization leads to the exposure of hydrophobic surfaces that drive membrane insertion (Iacovache et al., 2006). Pore formation renders the plasma membrane permeable to small ions but not proteins (Abrami et al., 2000). Specific cellular effects observed upon aerolysin treatment are release of calcium from the endoplasmic reticulum (ER); vacuolation of the ER (Abrami et al., 2000); and production of proinflammatory molecules such as tumor necrosis factor α (TNFα), interleukin 1β, interleukin 6, and prostaglandin E2 (Chopra et al., 2000; Galindo et al., 2004a). We show here that, by allowing the efflux of intracellular potassium, aerolysin triggers the activation of caspase-1. This cysteine protease is produced as a 45 kDa precursor that requires autocatalytic processing for activation.

Autoproteolysis depends on the formation of a large multiprotein oligomeric complex called the inflammasome (Martinon and Tschopp, 2005), which brings procaspase-1 molecules in close proximity of one another. Two types of inflammasomes have been reported, each containing a different member of the intracellular pattern recognition receptors, the so-called NLR (NACHT-LRR) family of proteins (Martinon and Tschopp, 2005). NLR members are typically composed of three domains: a leucine rich repeat (LRR) ligand-sensing domain, a NACHT oligomerization domain, and a C-terminal homotypic protein-protein interaction domain that can be either a caspase recruitment domain (CARD) or a pyrin domain. Recently some natural stimuli of the inflammasomes have been identified, such as uric acid crystals associated with Gout (Martinon et al., 2006), bacterial RNA (Kanneganti et al., 2006), extracellular ATP, the calcium channel affecting marine toxin maitotoxin (Mariathasan et al., 2006) and cytoplasmic flagellin (Franchi et al., 2006; Miao et al., 2006), leading to the view that inflammasomes are intracellular detectors of danger signals. Two NLR members can induce caspase-1 activation: NALP and IPAF. Genomic analysis has revealed 14 NALPs in the human genome, of which NALP1 and NALP3 have been best characterized and shown to be involved in caspase-1 activation (Chamaillard et al., 2003). The interaction of NALPs with caspase-1 is not direct and depends on the adaptor protein ASC (apoptosis-associated speck-like protein containing a CARD) (Martinon and Tschopp, 2005). IPAF, for which only one form has been reported, in contrast associates directly with caspase-1 via its C-terminal CARD domain (Martinon and Tschopp, 2005), although direct or indirect interactions with ASC may exist (Mariathasan et al., 2004; Sutterwala et al., 2006).

We here found that aerolysin-induced K^+ efflux triggers the assembly of IPAF and the NALP3 inflammasomes and the activation of caspase-1. Most importantly, we found that caspase-1 then induces the activation of the central regulators of membrane biogenesis, the sterol regulatory element binding proteins (SREBPs), which in turn promote cell survival upon toxin challenge. This study highlights that, in addition to its well-established role in triggering inflammation via the processing of the precursor forms of interleukins 1β, 18 (Fantuzzi and Dinarello, 1999), and 33 (Schmitz et al., 2005), caspase-1 has a broader role than previously appreciated, in particular linking the intracellular ion composition to lipid metabolic pathways.

RESULTS AND DISCUSSION

Pore Formation by Aerolysin Leads to the Activation of SREBPs

To study the cellular response to pore-forming toxins, we performed an RNA differential display analysis using the highly sensitive amplification of double-stranded cDNA end restriction (ADDER) fragments method (Kornmann et al., 2001). Chinese hamster ovary (CHO) cells were treated with low concentrations of proaerolysin (0.1 nM [Ratner et al., 2006]), conditions under which all cells excluded the DNA-intercalating agent propidium iodide for at least 10 hr (Figure S1A) (note that the protoxin is processed to aerolysin by cell-surface proteases, leading to pore formation). The ADDER analysis led to the identification of various differentially expressed genes, in particular the sterol regulatory element binding protein-2 (SREBP-2), which was upregulated after 3 hr (Figure S1B). SREBPs are membrane bound transcription factors that regulate the expression of genes harboring a sterol responsive element (SRE) in their promoter region and which are typically involved in cholesterol and fatty acid biosynthesis (for review see Goldstein et al., 2006). SREBPs initially reside in the ER. Release of the transcription factor domain from the membrane requires sequential proteolysis by two transmembrane enzymes, S1P and S2P, which reside in the Golgi apparatus. Cleavage of SREBPs thus requires transport from the ER to the Golgi, a step that is controlled by the escort protein SCAP (Goldstein et al., 2006). The following three major SREBP isoforms are known: SREBP-1a and -1c, which are encoded by a single gene and are preferentially involved in fatty acid metabolism, and SREBP-2, which is encoded by a distinct gene and controls cholesterol and lipid biosynthesis (Goldstein et al., 2006; Horton and Shimomura, 1999). Different stimuli have been reported to induced SREBP activation, including cholesterol depletion (Goldstein et al., 2006), ER calcium depletion (Lee and Ye, 2004), growth factors and insulin signaling (Demoulin et al., 2004; Nadeau et al., 2004), phagocytosis (Castoreno et al., 2005), and exposure of cells to hypotonic media (Lee and Ye, 2004).

Since regulation of SREBPs mainly occurs at the protein level rather than the transcriptional level, we tested whether aerolysin triggers SREBP-2 processing. Membrane and nuclear fractions were prepared from toxin-treated cells and probed by Western blotting using an antibody against the N terminus of SREBP-2. In untreated cells, full-length SREBP-2 (123 kDa) was found in the membrane fraction (Figure 1A), as expected since cells were grown in the presence of serum, the high-lipid content of which suppresses SREBP activation. Upon aerolysin treatment, the amount of full-length SREBP-2 decreased with time with the concomitant appearance of an ~60 kDa form in the nuclear fraction. Migration of SREBP-2 to the nucleus was confirmed by immunofluorescence microscopy (Figure 1B). Interestingly, the second SREBP isoform, SREBP-1, also underwent activation upon toxin treatment (Figure 1C). These results show that aerolysin caused the proteolytic processing and nuclear translocation of SREBPs.

We next measured the effect of aerolysin on the mRNA levels of two SREBP target genes: HMGCoA reductase and fatty acid synthase activated by SREBP-2 and SREBP-1 respectively (Horton and Shimomura, 1999). Both mRNAs increased upon toxin addition as shown by real-time PCR (Figures 1D and 1E, >3 fold after 3 hr). Also, the total cellular cholesterol increased by 25% to 30% after 3–5 hr toxin treatment (Figure 1F). Similar