Research Highlight

Molecular Suicide Notes: Last Call from Apoptosing Cells

Christoph Peter, Sebastian Wesselborg, and Kirsten Lauber*

Section of Molecular Gastroenterology, Department of Internal Medicine I, University of Tuebingen, Otfried-Mueller-Str. 10, D-72076 Tuebingen, Germany
* Correspondence to: Kirsten Lauber, Tel: + 49-7071-29-83137; Fax + 49-7071-29-4680; E-mail: kirsten.lauber@med.uni-tuebingen.de

Dying cells actively attract professional phagocytes by sending out soluble ‘find-me’ signals. The characterization and identification of the corresponding molecular entities have revealed that ‘find-me’ signals come in different flavors, including peptides, proteins, lipids and complex structures, such as apoptotic microblebs. With the very recent report by Elliott et al., nucleotides as a novel nuance enter the menu.

Apoptosis is the primary mechanism by which physiological cell death occurs during developmental processes and homeostatic cell turnover. Everyday this results in a considerable amount of dead cell mass that has to be removed. Otherwise, apoptotic cells proceed to the stage of secondary necrosis, losing the integrity of their plasma membrane, releasing intracellular contents and promoting the onset of chronic inflammation and autoimmunity. Indeed, non-engulfed apoptotic cells are rare to be seen in vivo. This is due to the fact that they are swiftly removed by phagocytosis-competent neighboring cells or by professional phagocytes, including macrophages and immature dendritic cells. But how do phagocytes track their prey? Since long, it has been postulated that dying cells secrete soluble mediators, so-called ‘find-me’ signals, which assist scavenger cells in navigating toward the sites of ongoing apoptosis. However, the corresponding molecular entities are still being discovered. In a recent report in Nature, Elliott et al. (2009) identified a new class of ‘find-me’ signals as the extracellular nucleotides ATP and UTP, which so far have been principally known for their role in energy metabolism, as monomeric building blocks of nucleic acids and as neurotransmitters.

Elliott et al. examined the migratory response of monocytes and macrophages toward culture supernatants of apoptotic cells in an in vitro transwell system and an in vivo mouse air pouch model. They observed the caspase-dependent release of soluble, chemotactically active mediators specifically recruiting monocytes and macrophages but not neutrophils in an early phase of apoptosis, in which the plasma membrane was still intact. These apoptotic cell-derived chemotactants were inactivated by digestion with nucleotide degrading enzymes, such as apyrase and CD39 (NTPDase-1), suggesting that nucleotides are crucially involved in this scenario. Follow-up experiments revealed that apoptotic culture supernatants contain approximately equimolar amounts of ATP and UTP, and that both nucleotides potently induce monocyte chemotaxis at concentrations similar to those found in the supernatants (upper nanomolar range). In contrast, supernatants of triton-lysed cells contained 2.5-fold more ATP than UTP. Hence, the equimolar release during apoptosis reflects either the consumption of ATP during apoptosis or the existence of mechanisms favoring the selective export of UTP. So far, only little is known about the controlled secretion of nucleotides across an intact plasma membrane. Whereas in neuronal cells, ATP is packaged within vesicles near the presynaptic membrane and upon stimulation is exocytosed into the synaptic space, in non-neuronal cells various membrane transport proteins or functionally characterized permeability pathways have been suggested to act as ATP channels, including some ATP-binding cassette-family transporters, volume-regulated anion channels, plasma membrane variants of the mitochondrial voltage-dependent anion channel, porins and maxianion channels (Fitz, 2007). Additionally, a strong and growing body of data indicate that ATP release from many cell types is mediated by so-called hemichannels composed of protein subunits from the well-characterized connexin family or the recently described pannexin family (Fitz, 2007). Future studies have to elucidate, which of these players are involved in the release of nucleotides during apoptosis.

In the second part of their work, Elliott et al. investigated which phagocytic receptor is involved in the sensing of extracellular nucleotides and mediates the subsequent migratory response. Pharmacological inhibition, RNA interference and genetic knockout experiments revealed that the purinergic G-protein-coupled receptor P2Y2, at least in part, contributes to ATP- and UTP-stimulated monocyte chemotaxis. To study the relevance of the P2Y2-nucleotide axis for apoptotic cell clearance in vivo, Elliott et al. finally employed a model of glucocorticoid-induced thymus atrophy. When administering nucleotide degrading apyrase or P2Y receptor blocking suramin, they observed a transient delay in thymus atrophy as measured by thymus cellularity and size. This was paralleled by a temporary accumulation of apoptotic cells.
suggesting that the macrophage-mediated clearance process was delayed. Additionally, an increased persistence of apoptotic cells in the thymi of P2Y2-deficient mice was to be observed. These findings are compatible with a scenario, in which extracellular nucleotides released by apoptosing thymocytes and P2Y2-dependent macrophage recruitment contribute to apoptotic cell clearance, at least in the context of glucocorticoid-induced thymus atrophy.

Extracellular nucleotides are not the only ‘find-me’ signals, which have been identified (Figure 1). Historically, the first apoptotic cell-derived attraction signal characterized was a covalent dimer of ribosomal protein S19 (dRP S19), followed by fragments of the human tyrosyl tRNA synthetase (TyrRS), thrombospondin 1 (TSP-1), the soluble IL-6 receptor (sIL-6R) and the CX3C chemokine fractalkine (FKN) (Lauber et al., 2004; Chalaris et al., 2007; Truman et al., 2008). Apart from these proteinaceous compounds, lipid ‘find-me’ signals, including lysophosphatidylcholine (LPC) and sphingosine-1-phosphate (S1P), and even complex structures, such as apoptotic microblebs, have been described (Segundo et al., 1999; Lauber et al., 2003; Gude et al., 2008). However, the identification of nucleotides in the context of immunologically silent apoptotic cell clearance comes as a surprise, since extracellular ATP has been well known as a classical danger signal. As such, it has been attributed a crucial role in alerting the immune system in the context of allergen-induced asthmatic airway inflammation and the T cell response against tumor cells stimulated to undergo an immunogenic form of cell death by anthracyclines and organoplatic compounds (Idzko et al., 2007; Ghiringhelli et al., 2009). In these studies, extracellular ATP has been reported to efficiently recruit and activate monocytes, macrophages, dendritic cells and intriguingly also neutrophils. Consequently, the question that arises is why Elliott et al. observed a specific invasion of monocytes and macrophages but not neutrophils. It might be due to the interplay of the complex mixture of ‘find-me’ signals present in the culture supernatants and in particular the recently identified neutrophil ‘keep-out’ signal lactoferrin (LTF), which accounts for this effect (Bournazou et al., 2009). Nevertheless, future studies have to clarify, whether other ‘find-me’ or ‘keep-out’ signals fine tune the danger signal function of extracellular nucleotides or, if not, in how far the release of ATP and UTP fits into the current concept that apoptotic cell clearance is anti-inflammatory.

Figure 1 Apoptotic cell-derived ‘find-me’ and ‘keep-out’ signals and their corresponding receptors. dRP S19, covalent dimer of ribosomal protein S19; FKN, fractalkine; LPC, lysophosphatidylcholine; LTF, lactoferrin; μBleb, microbleb; NTP, nucleoside-triphosphate; S1P, sphingosine-1-phosphate; sIL-6R, soluble IL-6 receptor; TyrRS, tyrosyl tRNA synthetase.
Furthermore, the relevance of the various ‘find-me’ signal-receptor systems for apoptotic cell clearance in different tissues and organs remains to be comparatively analyzed. This is a highly interesting question, especially in view of the delayed clearance model of autoimmunity (Schulze et al., 2008). Does the delay in apoptotic cell clearance observed in P2Y2 knock-out mice lead to the development of an autoimmune phenotype like it has been reported for mice deficient in G2A, the mediator of LPC-induced macrophage recruitment (Le, 2001; Peter, 2008)? Do patients with systemic lupus erythematosus display defects in nucleotide release during apoptosis or the phagocytic sensing mechanisms? If so, the interesting work by Elliott et al. does not only allow us an additional insight into the complex signaling mechanisms between dying cells and their scavengers but opens a putative therapeutic perspective for chronic inflammation and autoimmunity.

References


