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Editorial

dsDNA ASCs for caspase 8-mediated apoptosis

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In the dim and distant past of research, there was only place for one form of cell death. Necrosis was the only known way for cells to die, and pathologists generally defined all cells with a dying phenotype as necrotic. This restricted view was challenged in 1972 when Kerr *et al.*[1] defined apoptosis as a novel form of cell death, which was substantially different from necrosis, with regards to morphological features as well as most biochemical processes. While necrosis was considered an accidental form of cell death, often initiated by and leading to membrane rupture and associated inflammatory responses, apoptosis was found to follow largely a strictly regulated and programmed path, leading to an active cell destruction of the targeted cells in an immunologically silent manner. In particular, after realizing the excessive rates apoptosis may proceed in certain tissues at any given time without leaving notable traces, for example, as seen during thymic selection processes where 95% of all developing T cells are eliminated by apoptosis, it was considered the preferred default pathway of cell death induction.

More recent years have taught us, however, that necrosis may also proceed under certain circumstances in a rather organized and programmed manner and in particular in the absence of caspase activation.[2] While death receptor activation generally leads to apoptosis, or may even fail to promote cell death and rather activate various survival signals, inhibition of caspase activity can result in the activation of RIPK1 and 3, and subsequently to a programmed form of necrosis, termed necroptosis.[2] Why under certain conditions necroptosis, rather than the silent way to die via apoptosis, is preferred is presently largely unexplored. We may, however, speculate that sometimes dying with a big bang may be preferred when it may help to alarm protective immune responses thereby help other cells and tissues to survive.

Apparently, three ways to die are not enough. While some caspases, that is, caspase 2, 3, 7, 8, 9 and 10, have a critical role in the execution of apoptotic cell death, caspase 1 was considered solely important for cleavage and activation of the inflammatory cytokines IL-1β and IL-18. Thus, stimulation of immune cells, such as macrophages, by various kinds of danger signals generally leads to caspase 1-mediated maturation of IL-1β and associated inflammatory responses. In particular, in macrophages it was though also noted that excessive caspase 1 activation results in a most rapid form of inflammatory cell death closely resembling necrosis and thus termed pyroptosis.[3–5]

While the death-inducing signaling complex (DISC) and the apoptosome are the major activation platforms for apoptosis-related caspases, the inflammasome is generally accepted as the major activation mechanism of caspase 1.[4,5] The inflammasome is a multiprotein complex with often variable composition, generally consisting of a danger-, stress- or pathogen-sensing component, an adaptor protein and the proform of caspase 1. Controlled aggregation of these proteins results in close proximity-mediated proteolytic cleavage and activation of caspase 1. The adaptor protein ASC (apoptosis-associated speck-like protein containing a CARD) has a central role in the formation of the inflammasome. Activation of various Nod-like receptors (NLRs) or AIM2 (absent in melanoma-2) leads to the recruitment of ASC, which in turn binds and recruits caspase 1, eventually leading to IL-1β and IL-18 processing and associated inflammation. Excessive ASC-mediated caspase 1 activation appears to enhance inflammation by the rapid induction of pyroptosis,[3] in particular in pathogen-infected macrophages. As NLRs and AIM2 are often triggered by bacterial and viral products, for example, cytoplasmic dsDNA and bacterial toxins, pyroptosis may help to limit pathogen spreading and further alerts uninfected immune cells by the induction of inflammatory processes.

A recent study of Sagulenko *et al.*[6] in *Cell Death and Differentiation* now shows a yet different aspect of inflammasome-mediated cell death induction in macrophages. In the absence of caspase 1, activation of AIM2 by cytoplasmic dsDNA, or NLRP3 by the bacterial toxin nigericin leads to an ASC-mediated recruitment of caspase 8, and subsequent induction of apoptotic cell death, rather than pyroptosis (Figure 1). While these processes appear to occur also in the presence of caspase 1, caspase 1-mediated pyroptosis seems to be faster and dominant, and to obscure caspase 8-mediated apoptosis. Thus, ASC-mediated caspase 8 activation represents an alternative mechanism how cell death is induced in response to pathogen-associated triggers. Interestingly, caspase 1 and 8 recruitment and activation relies on different ASC domains. While the CARD domain is essential for caspase 1 recruitment, the pyrin domain of ASC appears to be critical to mediate caspase 8 activation and apoptosis. Thus, different parts of ASC transmit different forms of cell death. Also somewhat surprising is the observation that apparently caspase 8 is required for this form of apoptosis, while no need for either caspase 2 or 9 was observed. Similarly, ASC-mediated apoptosis was not blocked by Bcl-2

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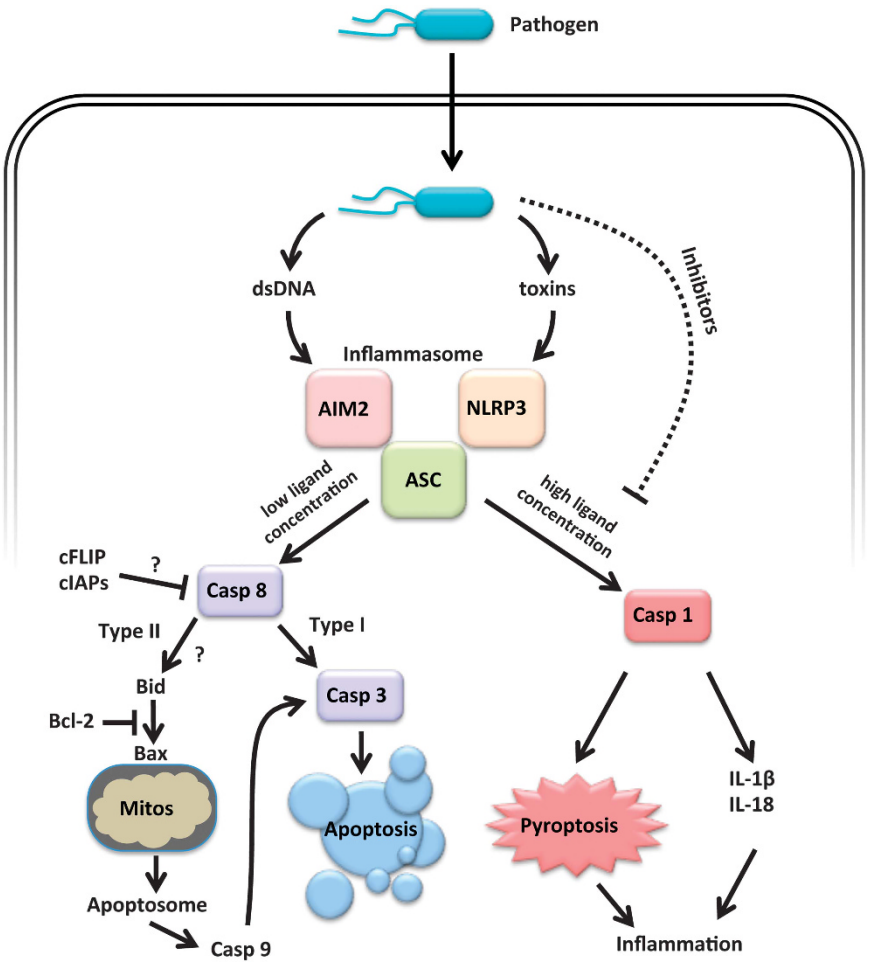

Figure 1 ASC-mediated switch between pyroptosis and apoptosis: intracellular pathogens and their products lead to activation of the ASC-dependent inflammasome. Strong inflammasome activation preferentially leads to caspase 1 recruitment and activation, and associated pyroptosis, IL-1β and IL-18 activation, and inflammation. Pathogen-derived inhibitors may prevent caspase 1 activation. Weak inflammasome activation promotes ASC-mediated caspase 8 recruitment and activation of the apoptotic pathway. Depending on the signaling cell type (type I or II), the mitochondrial signaling pathway may or may not be engaged

overexpression, suggesting that apoptosis does not proceed via the mitochondrial pathway. Thus, ASC-mediated caspase 8 activation seems to represent some kind of DISC, as observed for the CD95 and TRAIL receptor complex,[7,8] in the absence of any obvious participation of death receptors. The apparent absence of any contribution of the mitochondrial apoptosis pathway likely needs further confirmation. In analogy to the CD95-induced death receptor pathway, it may be feasible that ASC-mediated apoptosis in so-called type I cells directly leads to activation of effector caspases and apoptosis, and does not require amplification of the signal via Bid cleavage and activation of the mitochondrial apoptosis pathway. This may, however, be different in type II cells, which may depend on this amplification loop.[9] Along these lines is the finding that ASC-mediated apoptosis in certain tumor cells depends on the presence of Bax,[10] and is blocked by the absence of Bid or by Bcl-2/BclxL overexpression.[11] Alternatively, the mitochondrial contribution may depend on how the inflammasome and ASC-dependent apoptosis is triggered.

What are the decision-making signals that either lead to ASC-mediated activation of caspase 1 and pyroptosis, or to caspase 8 activation and apoptosis? While further studies will be required to illuminate these regulatory processes, it is feasible to suggest that factors affecting caspase 8 activation at the death receptor DISC may also affect caspase 8 activation at the inflammasome. Thus, high levels of cFLIP may interfere with caspase 8 maturation. Similarly, cellular inhibitors of apoptosis (cIAPs) positively and negatively regulate inflammasome activation and caspase 1 processing,[12] and also compromises caspase 8 activation at the death receptor DISC.[13] Thus, via ubiquitination of specific signaling components cIAPs may shift the inflammasome response towards inflammation and pyroptosis, or immunologically silent apoptosis.[14]

At the end, it remains to be asked why nature has invented a system that allows simultaneous induction of cell death by pyroptosis and associated inflammation, and apoptosis. Like necroptosis may be a backup mechanism of apoptosis, ASC-induced apoptosis may take over when pyroptosis is blocked, for example by pathogen-derived inhibitors. It is of interest that various bacteria, fungi and viruses have developed mechanisms that limit inflammasome and caspase 1 activation, and thereby likely also pyroptosis, evidently in an attempt to limit pathogen-eliminating protective immune responses.[15] Given the fact that the shift from caspase 1-mediated pyroptosis to

caspase 8-mediated apoptosis was largely dependent on the amount of cytoplasmic dsDNA, it is also feasible to suggest that pyroptosis and associated inflammation may be preferred when host cells are acutely invaded by intracellular pathogens, leading to high concentrations of cytoplasmic dsDNA, whereas apoptosis may serve to control latent and persistent infections where only low concentrations of pathogen-derived dsDNA or other bacterial products may be present in target cells. Last but not least, as ASC-mediated apoptosis proceeds in the absence of caspase 1, this mechanism may extend the role of inflammasome-triggered cell death to various cell types lacking caspase 1 expression, e.g. epithelial tumor cells. This novel finding by Sagulenko *et al.*[6] considerably changes our present view and understanding of which form of cell death has to be considered 'default' and 'backup', and which one is 'good' and beneficial for the host. Apparently, it is all a matter of the appropriate context.

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