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Editorial

Another twist in the on and off affair between cell suicide and inflammation

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When Kerr *et al.*[1] first adopted the term 'apoptosis' for cell suicide, they emphasised that apoptosis was stealthy, and not associated with inflammation, whereas when cells were killed, they exhibited a different appearance, termed 'necrosis', and this was accompanied by the signs of inflammation.

The first twist occurred with the cloning of the programmed cell death effector gene *ced-3* from *Caenorhabditis elegans* and the realisation that the cysteine protease it encoded was a homologue of mammalian interleukin-1β (IL-1β)-converting enzyme (now known as caspase 1).[2] Caspase 1 had been identified as the protease responsible for cleaving pro-IL-1β to generate the active cytokine,[3] which is one of the most pro-inflammatory known. Indeed, one of the original names for IL-1β was 'endogenous pyrogen'. As expression of human Bcl-2 in *C. elegans* had shown that apoptosis of mammalian cells and programmed cell death in the worm were carried out by the same, evolutionarily conserved mechanism,[4] it was therefore assumed that caspase 1 would have a major role in apoptosis in mammals.

As a result, the past exclusivity of apoptosis and inflammation was quietly forgotten, and a new paradigm that apoptosis and inflammation had a common evolutionarily link was established. Over-expression of caspase 1 in mammalian cells caused their death, and this could be blocked by the viral caspase 1 inhibitor CrmA.[5] Further reinforcement of this idea came from the phenotype of caspase 1 gene knockout mice, whose thymocytes were reported to be insensitive to apoptosis in response to ligation of Fas.[6]

However, the story took another twist when it was shown that it was caspase 8 rather than caspase 1 that was

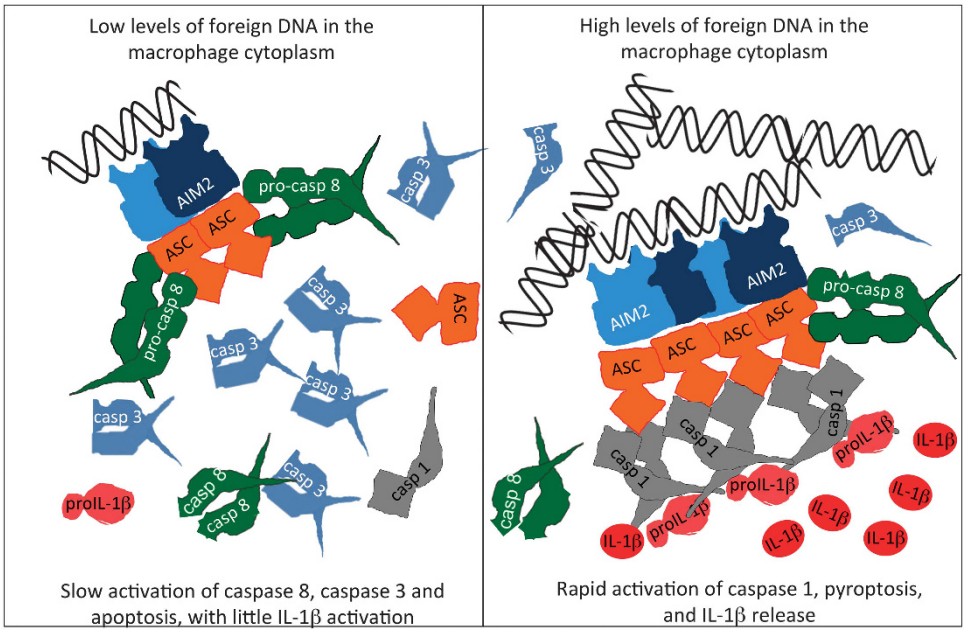

Figure 1 When a small amount of DNA is electroporated into the cytoplasm of macrophages (left panel) it is bound by AIM2, which triggers formation of an inflammasome by ASC. This activates caspase 8, which activates caspase 3 leading to the classic changes associated with apoptosis such as cleavage of I-CAD and CAD-mediated degradation of the nuclear DNA. When higher levels of DNA are present (right panel), AIM2 and ASC form an inflammasome much more rapidly, and it activates caspase 1 that processes pro-IL-1β and causes cell death characteristic of pyroptosis. One of the surprising findings of Sagulenko *et al.*[17] was that ASC can not only bind with procaspase 1 via their CARDs, but can also bind to procaspase 8 via pyrin–DED interactions

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responsible for Fas-induced apoptosis,[7,8] which suggested that caspase 1 was only involved in immune responses, and not in cell death.[9] This led to a new paradigm that there were two families of caspases: those involved in apoptosis (caspases 2, 3, 6, 7, 8, 9 and 10) and those involved in cytokine activation (caspases 1, 4, 5 and 11).

Recognition of the apoptosome, comprised of cytochrome c, Apaf 1, caspase 9 and caspase 3,[10] inspired naming of an analogous caspase 1-activating 'inflammasome' by Tschopp,[11] and the identification of signal transduction and adaptor proteins that regulate it, in particular the pyrin and CARD domain-bearing adaptor protein ASC/Pycard.[12]

The story turned once more, with a re-association of apoptosis and inflammation, when it was shown that ligation of Fas could trigger processing and release of IL-1β independent of caspase 1,[13] that caspase 8 could directly activate pro-IL-1β[14] and that in some cells stimuli (such as cytoplasmic DNA) could trigger activation of caspase 1 and simultaneously cause both IL-1β release and cell death. This form of pro-inflammatory cell suicide has been dubbed 'pyroptosis'.[15,16]

A paper in this edition of *Cell Death and Differentiation* advances the story even further by showing that the AIM2 and NLRP3 inflammasomes can trigger ASC to activate not only pyroptotic (IL-1β releasing) death pathways, but also an apoptotic pathway, and more surprisingly, caspase 8 is involved in both.[17]

Sagulenko *et al.*[17] looked at bone marrow-derived macrophages from normal and gene-deleted mice, and studied their responses to calf thymus or synthetic polydA:polydT DNA that had been electroporated across the plasma membrane. This treatment causes most wild-type macrophages to die within an hour.[18] Cytoplasmic DNA triggered the AIM2 inflammasome to activate caspase 8 and caspase 1, leading to cell death with features of both apoptosis (such as activation of caspase 3 and caspase-activated DNAse (CAD)-mediated DNA laddering) as well as pyroptosis (such as rapid loss of plasma membrane integrity and release of IL-1β).[17]

They found that lower concentrations of DNA led to pure apoptosis, whereas higher amounts caused pyroptosis as well. Once pyroptosis was triggered, it proceeded much more rapidly than apoptosis. Intriguingly, even when apoptosis was induced by low-dose DNA, caspase 9 (and hence the classic apoptosome) was *not* involved, but caspase 8 was implicated in both apoptosis and pyroptosis. Supporting this conclusion, procaspase 8 was found in association with the inflammasome adaptor protein ASC, bound via its DEDs to the pyrin domain of ASC, and knockdown of caspase 8 prevented electroporated DNA from killing caspase $1^{-/-}$ macrophages.

These results are consistent with a model in which AIM2 monitors the cytoplasm for the presence of foreign DNA, and if low levels are detected, an apoptosome is formed that contains ASC and procaspase 8 (Figure 1). This triggers activation of caspase 8 and caspase 3, which leads to all the changes that typify apoptosis, such as DNA ladder formation when caspase 3 cleaves I-CAD to release CAD. When higher levels of cytoplasmic DNA are present, AIM2 and ASC not only recruit caspase 8, but also recruit and activate caspase 1, which processes IL-1β and in addition causes rapid death by pyroptosis before many of the classic apoptotic changes have taken place.

Sagulenko *et al.*'s findings complement those by Pierini *et al.*,[19] who recently reported that in macrophages infected with *Francisella* bacteria, AIM2 and ASC could directly active caspase 8 to cause apoptosis, even when caspase 1 was deleted.

Although there are details still to be resolved, such as how the pyrin domains of ASC are able to bind to the DED domains of caspase 8, the overall picture is very satisfying, as it shows how a macrophage can make a graded response to an intracellular threat. These results also illustrate the strong biochemical link that exists between cell death and innate immunity, indicative of a shared evolutionary origin, with the 'apoptotic' caspase 8 being able to activate IL-1β, and the 'cytokine activator' caspase 1 being able to cause cell death.

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