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News and Commentary

Infection and the origins of apoptosis

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When did apoptosis evolve?

The ubiquity of apoptosis and the importance of its roles in differentiation, development and homeostasis are well described. But we have not come close to answering the fundamental questions of when, and under what circumstances, apoptosis first arose. Caspase-mediated apoptotic cell death is probably found in all cell types throughout the metazoa, although it is apparently absent from the fungi and the higher plants. Several of the key apoptosis regulators are found in *Caenorhabditis* (*Nematoda*), insects and vertebrates, and caspases have also been identified in *Asterina* (*Echinostomata*) and *Hydra* (*Cnidaria*).[1,2] These distributions, and other considerations, have led to the proposal that apoptosis (as defined by these molecular pathways) arose in one or more of the first multicellular animals, where it played, and continues to play, a role in assuring the 'social' interactions between cells, based on survival factor signaling.[3] However, although apoptosis is probably restricted to the animals, there are several signaling components (including relatives of the caspases) that appear to have counterparts in plants and fungi.[4] The question arises, then, as to whether apoptosis as we define it could have arisen in protists.

The notion that cell death in response to limited resources can help promote the survival of genetically related individuals seems, at first glance, to be reasonable. However, altruistic cell death (or any cell death) by definition favors individuals that have not died, and therefore the genetic pathways leading to death will tend to become mutated in 'selfish' individuals and the strategy of altruism at this level will not be stable. Any altruistic behavior will therefore be challenged by the rise of the 'egoists'.[5] There are several proposed solutions to the problem posed by altruism, but these tend to fail at the level of single celled organisms. These include a low mutation rate to limit the appearance of 'selfishness'.[5] Alternatively, selfishness in an individual can be recognized and effectively punished by non-cooperation, but this requires an ability to distinguish between individuals in the population and to predict their behavior. Of course, kin recognition can also permit altruism, but again, this is unlikely to be stable in single celled organisms.

So, if altruistic apoptosis evolved in protists, how could it be sustained and even favored? Several years ago we proposed that this might occur through a tight link between the components of apoptotic pathways and those of cell survival or proliferation mechanisms.[6] In this way loss of the apoptotic pathway would yield a cell that was incapable of replication. An example of this is the mitochondrial protein cytochrome *c*, where loss does not provide a survival advantage, despite the important role for this protein in apoptosis. However, it is now clear that many of the components of the apoptotic pathways are not essential for other functions, and even the role of cytochrome *c* may not be conserved throughout the metazoa. There is an interesting alternative, however, that not only creates conditions for stable altruistic behavior but also provides a basis for programed cell death in unicellular organisms. This alternative may hold the key for the interesting, and sometimes perplexing, connections between the molecular events of apoptosis and those of inflammatory responses.

Intracellular parasitism as a selection for altruistic apoptosis

Intracellular parasitic organisms (viruses, bacteria, fungi and protozoa) invade cells in order to exploit cellular resources and reproduce, usually killing the host cell in the process. The progeny of such a parasitic organism will then invade related cells to perpetuate the parasites' life cycle. If a cell can detect that such an infection has occurred and that it is therefore fated to die, then by engaging a mechanism for more rapid cell death it will halt reproduction of the parasite and prevent its lethal spread to other related individuals (keeping in mind that the most likely targets of a specialized parasite will be individuals that closely resemble the initial host). In this scenario, there is no benefit to being an 'egoist': any individual that is infected by a parasite that selfishly decides not to undergo rapid death will nevertheless die, and by allowing the parasite to reproduce, a cell will endanger its clone mates and relatives as well. Thus, relatedness between the altruist and those that benefit is effectively monitored by the parasite (not the individuals themselves) and similarly, the 'punishment for betrayal' is carried out by the parasite as well. Active cell death in response to infection essentially becomes one of the earliest types of immunity.

If this is so, we may expect that aspects of the molecular pathways involved diverged towards more sophisticated roles in the immune responses of different organisms. Since host-parasite interactions are dynamic, it should also be expected that parasites would evolve mechanisms to prevent such active cell death, and thereby promote their survival and replication. Evidence for both of these predictions is discussed further below.

Currently, however, there is no evidence that strongly supports the presence of a molecular pathway mediating apoptosis in unicellular organisms that is triggered in response to infection. Some protists have been observed to undergo cell death with the superficial characteristics of apoptosis,[7–11] but it remains to be seen whether the mechanisms are related to those of metazoans. Nevertheless, we can take advantage of the principle of parsimony to defend our position; that is, by comparing organisms in different phyla (or kingdoms) conclusions can be drawn about the characteristics of cell death in common ancestors. As we learn more about the pathways of apoptosis in diverse organisms, these characteristics will clarify.

Apoptosis and immunity

An important prediction derived from the above considerations is that the molecular pathways involved in apoptosis, and those involved in the response to infection, will overlap – i.e. share molecular features originating from a common antecedent. There is no need that this should have arisen in unicellular organisms for this to be the case, unless parallels can be seen across kingdoms; however, this is a possibility, as we shall see.

One of the most obvious examples of the overlap between apoptosis and the immune response is the processing of cytokines by caspases. Mammalian caspase-1, which may not be involved in apoptosis, is clearly required for the processing and secretion of interleukin-1 and interleukin-18, both of which participate in inflammatory responses to infection.[12,13] Similarly, caspase-3, the prime apoptotic executioner caspase, may be involved in the processing and secretion of interleukin-16, which acts as a chemotactic factor for T lymphocyte recruitment.[14] This function of caspases extends to insects; the caspase DREDD is required for the anti-bacterial response in *Drosophila*,[15] and appears to play a fundamental role in the processing and activation of Relish,[16] a member of the Nuclear Factor-κB (NF-κB) family of transcription factors.

There are multiple parallels between apoptotic pathways and the regulation of NF-κB, the factor centrally involved in the response to infection in both insects and vertebrates. Proteins involved in caspase activation contain related domains; either CARD (caspase recruitment domain) or DED (death effector domain). However, these domains are also associated with activation of NF-κB. For example, the CARD-containing proteins Bcl-10 (a.k.a. Ciper, Clap, or Carmen) and Rip2 (also called Rick or Cardiac) potently activate NF-κB. These may connect the mammalian Toll-like receptors – cell-surface molecules that recognize components of bacteria – to the NF-κB response (Figure 1)[17] Ligation of these receptors can also trigger apoptosis via a different pathway.[18] Similarly, the DED-containing protein RIP activates NF-κB in response to ligation of the TNF receptor,[19] and again, this receptor can trigger apoptosis via a different pathway.

Intracellular bacteria can trigger NF-κB-mediated gene activation through interaction with another CARD-containing protein, NOD1 (also called CARD4), which appears to be required for the intracellular response (with NF-κB activation) to bacterial lipopolysaccharides[20] or *Shigella* infection.[21] Recently, mutations in a related molecule, NOD2, were implicated in susceptibility to Crohn's disease, an inflammatory bowel disease.[22] Most strikingly, the domain structure of the NOD proteins, which in addition to a CARD contain a nucleotide binding domain and a leucine rich repeat domain, is homologous to a family of proteins in plants that are involved in host defense.[23] The plant hypersensitivity response involves the death of infected cells in response to infection, which has the effect of blocking systemic dissemination of the pathogen. A caspase related protein, metacaspase, has also been identified in plants[24] and if this is similarly involved in host defense via cell death, then the scenario we suggest above may be robust.

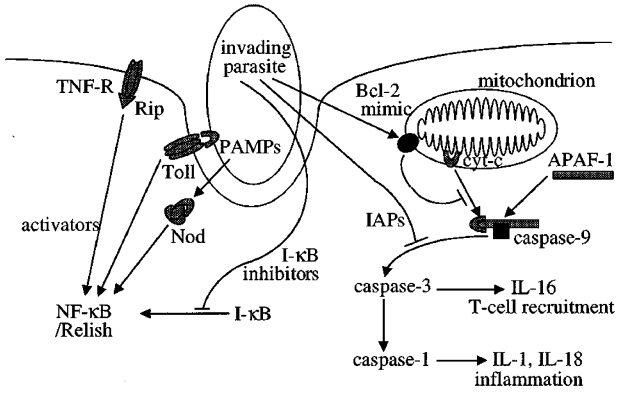

Figure 1 Apoptosis in the host-parasite interaction. Host cell invasion by parasitic organisms could, through the pathogen associated molecular pattern (PAMP) molecules, trigger NF-κB-family activation via Toll-like receptors or Nod-like proteins. NF-κB-family proteins, together with caspase-activated interleukins, help mediate 'immunity' to pathogens. In turn, pathogens take one or more of a number of anti-apoptotic actions to ensure residence in their host cell is not terminated prematurely. These may include inhibitors of NF-κB pathways, IAPs to block caspases and Bcl-2 mimics to block release of cytochrome-*c* (the mitochondrial PAMP)

Another prediction of the scenario we have suggested, linking infection with the evolution of apoptosis, is that infectious organisms will develop mechanisms to disable the apoptotic machinery of their host cell as a means of countering this defensive response. This phenomenon has been well described for infections with viruses,[25] bacteria,[5] fungi[26] or protozoa.[27,28] While in many cases the mechanism for resistance to apoptosis is unknown, in others there appear to be a variety of ways in which the parasite ensures cellular survival. These include the production of molecules that resemble and mimic Bcl-2,[29] the expression of inhibitors of death receptor signaling,[30,31] the generation of IAPs and other caspase inhibitors[32–34] and interference with NF-κB pathways[35,36] (Figure 1). Other mechanisms undoubtedly exist.

Do mitochondria trigger the intracellular response to infection

There is another consequence of the scenario linking infection to apoptosis that leads to some intriguing questions. If the

process of active cell death in response to infection is ancestral to the emergence of the eukaryotes, then the invasion by proto-mitochondria (as well, perhaps, as proto-chloroplasts and apicoplasts) into primitive eukaryotes should be capable of triggering the death of the cell. Clearly, at some point, this did not happen and symbioses arose. Nevertheless, intracellular sensors in the cytosol, capable of recognizing pathogen associated molecular patterns (analogous perhaps to the extracellular recognition of such PAMPs by the Toll-like receptors[37,38]) would trigger apoptosis when signals sequestered by the symbiont were released. This may represent the origins of the cytochrome *c* Apaf-1 interaction, and perhaps that of other mitochondrial components with other signaling pathways in the cytosol. These PAMPs need not be protein, and we cannot exclude that changes in mitochondria participate in the function of Ced-4 in *C. elegans* or ARK in *Drosophila*. Mammalian Bcl-2, which functions to regulate the release of the cytochrome *c* PAMP during apoptosis in mammals, also blocks cell death in *C. elegans* and *Drosophila*.[39]

Did mitochondria contribute the Bcl-2 family proteins to the apoptotic machinery of the eukaryotic cell as a mechanism for evasion of the infection-induced death response? Did the cell incorporate the regulation of infection signals from mitochondria into the regulated cell death apparatus of the cell? And did, therefore, infection (and the monitoring of host relatedness that is implied by host specificity) drive the evolution of apoptosis in unicellular organisms, providing the route to social control of cell life and death that permitted these organisms to eventually become multicellular?

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