Cell Death and the Caspase Cascade

Stephen M. Schwartz, MD, PhD

The article by Yaoita et al in this issue of Circulation is the first of what will indubitably be many articles on the role of the caspases in cell death. This is an important event. Until 10 years ago, we could not define death at all. Instead, we relied on the process of necrosis, the decay of the cell after its death, as a way to tell us that cells had died. Typical experiments involved applying a death stimulus for different time periods and then waiting hours or days to see if the death stimulus had effected a critical “point of no return.”

Another reason for believing in the caspases as a likely therapeutic target is the recent evidence that cells not only have caspases, they also have proteins able to inhibit the caspases or prevent their activation. The serine protease coagulation and complement cascades are regulated by antiproteases, including proteases that digest and inactivate other proteases, allosteric modifiers of protease activity or irreversible protease substrates (serpins) that bind and inactivate enzymes. Examples of similar “anti-caspases” are now emerging for the caspases (Table).

The simplest category of inhibitors may be substrates themselves. Tatsuta et al made the intriguing observation that cytoplasmic interleukin-1β (IL-1β), the prototypical substrate of the caspases, can act to inhibit Fas-mediated cell death. Although this might be an artifact of IL-1β secretion, it is worth considering the possibility that low levels of turnover of substrate regulate the activity of the low abundance, long-prodomain caspases and account for the need for activation of downstream caspases before death can occur.

Another category of caspase inhibitors depends on the activation mechanism for the long-prodomain caspases. Caspases 8 and 10 are activated by interacting with a death adapter protein, FADD. FADD aggregates these “signaling” caspases onto the Fas receptor after the receptor aggregates as a result of interacting with its ligand. For example, sentrin is a protein that binds domains on Fas but not FADD. Sentrin may inhibit FADD-dependent death by preventing aggregation of FADD on activated Fas and secondarily inhibiting recruitment of caspase 8.

Like the coagulation enzymes, the caspases also appear to be regulated by serpins. Viral serpins that enhance viral survival by inhibiting caspases include p35, a general inhibitor of caspases by viruses in insect cells, and CrmA, produced by the cowpox virus in mammalian cells. Recently, an endogenous, nonviral serpin for the caspases has been identified in mammalian cells, proteinase inhibitor 9 (PI9). Sprecher and collaborators used serpin homology to clone PI9. Other members of this new family of nonsecreted, cytoplasmic serpins have been serine protease inhibitors; however PI9 is a CrmA homolog. The selectivity of PI9 could be relevant to recent observations that different caspases, especially the long prodomain caspases including caspases 2, 8, 9, and 10, are involved in different types of cell death. Because PI9 can inhibit interleukin-1-converting enzyme (ICE), it is possible that it plays a role in inhibition of the ICE-like caspase believed to be required to activate mitochondria.

Recently, Schonbeck et al reported an as-yet-unidentified protease inhibitor capable of blocking caspase 1 (ICE) in smooth muscle but not endothelial cytoplasm.

The next category of caspase inhibitors, called IAPs, were also first recognized as viral proteins. Recently, however, mammalian IAPs have been recognized as well. Binding of
IAPs to the TRAF molecules in the death receptor complexes suggested a role at the level of the initial activation of the long-prodomain caspases; however, a recent paper by Deveraux et al.\(^{20}\) found that a mammalian X-linked IAP, XIAP, inhibited death by binding to caspases 3 and 7. Intriguingly, this effect was relatively specific for these terminal caspases. XIAP did not activate caspases 8, 6, or 1 even at 50-fold excess, at least against the substrate tested. This suggests that XIAP is an inhibitor of specific caspases. The mechanism of protease inhibition is not apparent, and it is not known whether this effect is limited to this one member of the IAP family.

The final category of caspase inhibitors identified first in viruses are called “FLIPs.” The FLIPs were recently discovered by Thome et al.\(^{21}\) and Hu et al.\(^{22}\) as viral proteins with homology to the DED of caspase 8 (FLICE). Death effector domains are the domains caspases use to aggregate to one another and to FADD. Like DED constructs of caspase 8, the viral FLIPs act as dominant negatives for FADD-mediated death apparently by acting as competitors for binding of the prodomains of caspase 8 or 10 and thus blocking Fas-mediated apoptosis. In recent months, eight different labs including our own have cloned cellular homologs of the FLIP gene.\(^{23}\) We called this cellular FLIP “MRIT.” Intriguingly, MRIT abundance is very high in myocardium.

Until this point, I have focused on the anticaspases as antiapoptotics. However, most readers are likely to be more familiar with another category of antiapoptotic genes, the “Bcl2” family. This is the same family as the \(ced-9\) gene already referred to in my comments about Horvitz’s work in \(C.\) \(elegans.\) Bcl2 members do control death, and \(ced-9\) appears to be mainly antiapoptotic. The mechanism for this action is confusing because Bcl2 homologs can also stimulate cell death. The proapoptotic Bcl2 homologs are believed to possess this activity via their ability to increase mitochondrial permeability, releasing the cytochrome \(c\). Bcl2 and its proapoptotic relatives seem to determine mitochondrial permeability by competing with each other in the formation of mitochondrial pores or perhaps by interacting with other molecules, including the mammalian \(ced-4\), to control caspase activation (see below). Cytochrome \(c\) in turn activates a cytoplasmic molecule that we now know is the mammalian equivalent of Horvitz’s third gene, \(ced-4\). \(ced-4\) functions, when complexed with cytochrome \(c\), to activate the effector caspases.\(^{24–34}\) In summary, we are beginning to see a central pathway of death that centers on the caspase cascade.

In this regard, the article by Yaoita et al.\(^{1}\) is likely to be the first of many exploring a new therapeutic direction. I would suggest there are three key areas to consider:

1. Tissue specificity. The first generation of drugs are very nonspecific, and we know little about their possible toxicity, even in animals.

2. Therapeutic efficacy. It is important to remember that caspases are not synonymous with death. Although death may be mediated in many situations by the effector caspases, it is likely that not all death occurs this way and equally likely that caspases play a role in cellular events in addition to death itself. An intriguing example of the latter may be the recent paper by Rodriguez et al.\(^{35}\) Normally, infusion of Fas-activating anti-

### Anticaspases

<table>
<thead>
<tr>
<th>Substrates in excess</th>
<th>Interleukin 1β</th>
<th>Viral Interactions</th>
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<tbody>
<tr>
<td>DD competitor</td>
<td>Senrin</td>
<td>Caspase 1</td>
</tr>
<tr>
<td>Cytoplasmic serpins</td>
<td>PAI-2</td>
<td>Fas &amp; TNF R DD domains, but not other DD</td>
</tr>
<tr>
<td></td>
<td>PI-9</td>
<td>Unknown protease</td>
</tr>
<tr>
<td>Caspase-binding inhibitors</td>
<td>c-IAPs X-IAP</td>
<td>Caspase 1</td>
</tr>
<tr>
<td></td>
<td></td>
<td>Csm A, p35, SPI-2</td>
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<tr>
<td>Dominant negative DED caspase</td>
<td>MRIT (aka CASPER, I-FLICE, c-FLIP)</td>
<td>Caspases, BCLx, BCLs, TRAFs, FADD</td>
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<td></td>
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<td>v-FLIP</td>
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\(\text{TNF}\) indicates tumor necrosis factor; \(\text{PAI-2}\), plasminogen activator inhibitor-2.
bodies kills an animal because the liver is very sensitive to Fas activation. However, when these investigators protected the liver with an antiapoptotic transgene, the mice still died. The mice showed no morphological indication of apoptosis, raising the intriguing possibility that death may have occurred from a sublethal activation of the caspase pathway (at least, sublethal at the level of the individual cell).

3. Finally, as seen in the article by Yaoita et al in this issue of Circulation, the protective effect of ZVAD-fmk was not total. Moreover, even the morphological evidence of an antiapoptotic effect, measured by the terminal deoxynucleotidyl transferase–mediated dUTP-biotin nick end labeling assay, correlated poorly and was much more marked than the more functional measure of infarcted area. We do not know whether this is an issue of dose or an issue of the duration of injury versus the duration of drug.

References


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