A time to kill: viral manipulation of the cell death program

Stewart Hay and George Kannourakis

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Many viruses have as part of their arsenal the ability to modulate the apoptotic pathways of the host. It is counter-intuitive that such simple organisms would be efficient at regulating this the most crucial pathway within the host, given the relative complexity of the host cells. Yet, viruses have the potential to initiate or stay the onset of programmed cell death through the manipulation of a variety of key apoptotic proteins. It is the intention of this review to provide an overview of viral gene products that are able to promote or inhibit apoptotic death of the host cell and to discuss their mechanisms of action. It is not until recently that the depth at which viruses exploit the apoptotic pathways of their host has been seen. This understanding may provide a great opportunity for future therapeutic ventures.

Introduction

Programmed cell death (PCD), as its name suggests, is an orchestrated biochemical process that leads ultimately to the demise of the cell. Included under this title of PCD are three broad categories by which a cell may die. Firstly and most typically is apoptosis, characterized frequently by chromatin condensation, phosphatidylserine exposure, cytoplasmic shrinkage, membrane blebbing and caspase activation. Secondly, in a process quite analogous, is ‘apoptosis-like PCD’, which presents with some of the apoptotic features but lacks the densely packed chromatin. The third category is ‘necrosis-like PCD’, which is typified by the general absence of chromatin condensation but is distinguished from necrosis (death resulting from injury) through its use of a signalling pathway (Leist & Jaattela, 2001; Shi, 2001; Tilly, 2001).

Apoptosis is the best-characterized form of PCD. It is a crucial component for normal multicellular life, playing a key role in development and immunity.

It is natural to expect viruses to have the ability to affect the apoptotic process within a host cell. This expectation is likely, given that, constitutively, virus survival is dependent upon the effective exploitation of the existing cellular machinery. Indeed, a virus can benefit and may instigate either promotion or inhibition of apoptosis, but often these parasites are greatly harmed by the natural apoptotic action elicited by the host. In response to a virus infection, the host produces an array of proteins, including cytokines and proteases. It utilizes the action of macrophages, natural killer cells, cytotoxic and helper T cells, neutrophils and B cells. Furthermore, in the infected site itself, the cells may respond through the swift signalling potential of interferon (IFN) (Roulston et al., 1999; Stark et al., 1998). Undoubtedly, it is a significant achievement for a virus to make its way through the intricate defence network of the host to establish an infection.

Collectively, apoptotic pathways contain many individual steps. A virus may need to influence only a single point of the process to affect the onset or progress of the natural cell death program. There are, however, strategies other than simple biochemical manipulation that viruses use to overcome the hindering effects of apoptosis. A virus may multiply rapidly to produce many virions before an effective immune response can be mounted. This approach is exhibited by most RNA viruses, including vesicular stomatitis virus and influenza virus (Koyama, 1995; Kurokawa et al., 1999). Another strategy available to viruses is that of a cryptic infection. In this situation, a virus may infect a cell and remain undetected, thus avoiding host cell destruction and allowing a productive infection (Di Rosa & Barnaba, 1998; Paroli et al., 2000).

Although the benefits to a virus in avoiding the apoptotic process are obvious, the onset of PCD is, in some cases, also advantageous. In such situations, the apoptotic demise of a cell results in the formation of small membrane-bound entities known as apoptotic bodies. These bodies pinch off from the dying cell and are consumed by the phagocytic action of neighbouring cells. This engulfment provides a means for the dissemination of the virus without initiating a concomitant host response, which would follow the release of the progeny into the extracellular fluid (Teodoro & Branton, 1997a). This
### Table 1. Viruses that produce apoptosis-inducing proteins

A virus infection will generally elicit an immune response resulting in apoptosis (Thompson, 1995).

<table>
<thead>
<tr>
<th>Virus</th>
<th>Pro-apoptosis gene(s) or protein(s)</th>
<th>Mechanism of action</th>
<th>Apoptosis induction seen in</th>
<th>Recombinant virus lacking gene or active protein*</th>
<th>Reference</th>
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<tbody>
<tr>
<td><strong>Adenoviridae</strong></td>
<td></td>
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<tr>
<td>Adenovirus</td>
<td>E1A</td>
<td>Associates with the pRb/p300 family and induces p53-dependent apoptosis</td>
<td>Many cancer cells</td>
<td>+</td>
<td>Heise et al. (2000); Lowe &amp; Ruley (1993)</td>
</tr>
<tr>
<td></td>
<td>E4orf4</td>
<td>Protein cooperates with E1A to promote apoptosis in the absence of p53</td>
<td>Mouse embryo fibroblast-derived cells</td>
<td>+ (E1 and E4)</td>
<td>Marcellus et al. (1996b)</td>
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<td></td>
<td></td>
<td>Interacts with protein phosphatase 2A (PP2A) to induce apoptosis</td>
<td>H1299</td>
<td>−</td>
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<td></td>
<td></td>
<td>Utilizes caspase 8/FADD pathway (cell specific)</td>
<td>293T</td>
<td>−</td>
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<tr>
<td><strong>African swine fever-like virus</strong></td>
<td>5EL</td>
<td>Functional IκB homologue, downregulates NFκB gene expression</td>
<td>−</td>
<td>+</td>
<td>Neilan et al. (1997)</td>
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<td><strong>Circoviridae</strong></td>
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<tr>
<td>Chicken anaemia virus</td>
<td>Apoptin (VP3)</td>
<td>Upstream caspase activation not required</td>
<td>Many transformed and tumour cell lines</td>
<td>−</td>
<td>Jeurissen et al. (1992)</td>
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<td></td>
<td></td>
<td>Apoptosis Bcl-2 and p35 inhibitable</td>
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<td><strong>Flaviviridae</strong></td>
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<tr>
<td>HCV</td>
<td>Core protein</td>
<td>Core protein binds to cytoplasmic domain of TNF</td>
<td>BC10ME, HepG2 and HeLa</td>
<td>−</td>
<td>Zhu et al. (1998)</td>
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<td></td>
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<td>Receptor 1-sensitization to TNF-induced apoptosis</td>
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<td><strong>Pestiviridae</strong></td>
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<tr>
<td>Pestivirus</td>
<td>E”ns</td>
<td>Inhibition of protein synthesis (glycoprotein with RNase function)</td>
<td>Lymphocytes in many species</td>
<td>+</td>
<td>Bruschke et al. (1997); Meyers et al. (1999); Schneider et al. (1993)</td>
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<td><strong>Hepadnaviridae</strong></td>
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<tr>
<td>HBV</td>
<td>pX</td>
<td>Sequesters p53 and facilitates apoptosis induction</td>
<td>Chang and HepG2</td>
<td>+</td>
<td>Lara-Pezzi et al. (1998); Su &amp; Schneider (1997); Su et al. (2001); Wang et al. (1995)</td>
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<td></td>
<td></td>
<td>Upmodulates TNF</td>
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<td><strong>Papovaviridae</strong></td>
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<tr>
<td>Human papillomavirus</td>
<td>E2</td>
<td>Regulates the transcription of E6/E7 and facilitates apoptosis, p53-dependent pathway</td>
<td>HeLa</td>
<td>+</td>
<td>Desaintes et al. (1997, 1999); Webster et al. (2000)</td>
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<td></td>
<td>E7</td>
<td>Involved with cell cycling and interacts with the pRb family and induces apoptosis</td>
<td>Mouse lens</td>
<td>+</td>
<td>Pan &amp; Griep (1995); Zwierschke &amp; Jansen-Durr (2000); Iglesias et al. (1998)</td>
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Table 1 (cont.)

<table>
<thead>
<tr>
<th>Virus</th>
<th>Pro-apoptosis gene(s) or protein(s)</th>
<th>Mechanism of action</th>
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<td>Papovaviridae</td>
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<tr>
<td>SV40</td>
<td>Large T</td>
<td>Blocks function of pRb (retinoblastoma) and p53 Apoptosis induction dependent on pRb binding</td>
<td>Mouse lens</td>
<td>+</td>
<td>Fromm et al. (1994); Kolzau et al. (1999); McCarthy et al. (1994)</td>
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<tr>
<td>Retroviridae</td>
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<tr>
<td>gp120/gp41 Vpr Nef</td>
<td>Syncytium formation and CD4 cross-linking Caspase-8-mediated pathway and modulated by the Bcl-2 family Increased expression of TNF on cell surface</td>
<td>MOLT4-T4 and H9 SupT1 and HeLa II-23.D7</td>
<td>– + –</td>
<td>Laurent-Crawford et al. (1993, 1995); Maldarelli et al. (1995); Sylwester et al. (1997) Chang et al. (2000); Conti et al. (2000); Patel et al. (2000); Stewart et al. (1997)</td>
<td>Lama &amp; Ware (2000)</td>
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<td>Togaviridae</td>
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<tr>
<td>Semliki Forest virus</td>
<td>Not known</td>
<td>Induces p53-independent apoptosis from the non-structural part of the genome</td>
<td>H358a and neurons</td>
<td>+</td>
<td>Allsopp et al. (1998); Murphy et al. (2000)</td>
</tr>
<tr>
<td>Sindbis virus</td>
<td>E1 and E2</td>
<td>Virus entry – initiates sphingomyelin degradation and ceramide release</td>
<td>AT3</td>
<td>–</td>
<td>Jan et al. (2000); Joe et al. (1998)</td>
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<tr>
<td>Rubella virus</td>
<td>Capsid</td>
<td>Endoplasmic reticulum-localized factor</td>
<td>Vero and RK13</td>
<td>–</td>
<td>Duncan et al. (2000); Law et al. (2001); Pugachev &amp; Frey (1998)</td>
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</table>

* A virus that has had a recombinant constructed that lacks the ability to produce an active form of this protein is indicated by ‘—’, whereas a recombinant virus that lacks the ability to produce an active form of this protein is indicated by ‘+’.
Table 2. Viruses that produce apoptosis-inhibiting proteins

<table>
<thead>
<tr>
<th>Virus</th>
<th>Anti-apoptosis gene(s) or protein(s)</th>
<th>Mechanism of action</th>
<th>Apoptotic stimulus</th>
<th>Recombinant virus lacking gene or active protein*</th>
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<tr>
<td>Adenovirus</td>
<td>E1B-19K</td>
<td>Bcl-2 type – Inhibits E1A, Fas, TNF-induced apoptosis specific form of Bax – caspase activation stopped before caspase-9 but after caspase-8</td>
<td>TNF-α</td>
<td>+</td>
<td>Farrow et al. (1995); Marcellus et al. (1996a); Perez &amp; White (2000)</td>
</tr>
<tr>
<td></td>
<td></td>
<td>Binds to nuclear lamins</td>
<td>E1A-induced p53-dependent</td>
<td>–</td>
<td>Rao et al. (1997)</td>
</tr>
<tr>
<td></td>
<td>E1B-55K</td>
<td>Binds to p53 and functionally inactivates it</td>
<td>E1A-induced p53-dependent</td>
<td>–</td>
<td>Teodoro &amp; Branton (1997b); White et al. (1992); Yew et al. (1994)</td>
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<tr>
<td>E3-6.7</td>
<td></td>
<td>Complexes with 10.4 and 14.5 resulting in downmodulation of TRAIL receptor 1 and 2</td>
<td>TRAIL and FasL</td>
<td>+</td>
<td>Benedict et al. (2001)</td>
</tr>
<tr>
<td>E3-10.4</td>
<td></td>
<td>Inhibits E1A or TNF-induced apoptosis</td>
<td>TNF-α</td>
<td>+</td>
<td>Gooding et al. (1988, 1991)</td>
</tr>
<tr>
<td></td>
<td></td>
<td>Inhibits TNF-induced release of arachidonic acid</td>
<td>TNF-α</td>
<td>+</td>
<td>Krajcsi et al. (1996)</td>
</tr>
<tr>
<td></td>
<td></td>
<td>Inhibits TNF-induced transfer of PLA2 to cell membrane</td>
<td>TNF-α</td>
<td>+</td>
<td>Dimitrov et al. (1997)</td>
</tr>
<tr>
<td>E3-14.5</td>
<td></td>
<td>Decreased presentation of Fas on the cell surface</td>
<td>Anti-Fas</td>
<td>+</td>
<td>Shisler et al. (1997)</td>
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<td></td>
<td>Inhibits E1A or TNF-induced apoptosis</td>
<td>TNF-α</td>
<td>+</td>
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<td>TNF-α</td>
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<td>TNF-α</td>
<td>+</td>
<td>Gooding et al. (1990)</td>
</tr>
<tr>
<td>E3-14.7K</td>
<td></td>
<td>Caspase pathway – caspase-2 and -8</td>
<td>TNF-α</td>
<td>–</td>
<td>Li et al. (1998)</td>
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<td></td>
<td></td>
<td>Inhibits TNF-induced release of arachidonic acid</td>
<td>TNF-α</td>
<td>+</td>
<td>Krajcsi et al. (1996)</td>
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<td><strong>African swine fever-like viruses</strong></td>
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<tr>
<td>ASFV</td>
<td>LMWS-HL/A179L</td>
<td>Bcl-2-related protein</td>
<td>Protein kinase (p68)</td>
<td>–</td>
<td>Afonso et al. (1996); Brun et al. (1996, 1998); Neilan et al. (1993); Revilla et al. (1997)</td>
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<tr>
<td>A224L</td>
<td>IAP-related protein</td>
<td></td>
<td>TNF-α and cycloheximide or staurosporine</td>
<td>+</td>
<td>Chacon et al. (1995); Nogal et al. (2001)</td>
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<td>Virus</td>
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<td>Mechanism of action</td>
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<td>Baculovirus</td>
<td>P35</td>
<td>Caspase inhibitors 1, 3, 6–8 and 10</td>
<td>Staurosporine and extracellular potassium</td>
<td>+</td>
<td>Bertin et al. (1996); Clem et al. (1991); Viswanath et al. (2000); Zhou et al. (1998)</td>
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<tr>
<td>B. P35</td>
<td></td>
<td>Inhibits oxidative stress-induced apoptosis</td>
<td>H$_2$O$_2$</td>
<td>+</td>
<td>Sah et al. (1999); Crook et al. (1993); Deveraux et al. (1997)</td>
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<td><strong>Herpesviridae</strong></td>
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<td>Bovine herpesvirus-4</td>
<td>BORFE2</td>
<td>Inhibits Fas and TNF-induced apoptosis. Contains death effector domains and interacts with caspase-8</td>
<td>Fas and TNF receptor 1</td>
<td>–</td>
<td>Wang et al. (1997)</td>
</tr>
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<td>EBV</td>
<td>BHRF1</td>
<td>Bcl-2-related protein</td>
<td>Low serum, ionomycin, cisplatin and etoposide</td>
<td>–</td>
<td>Henderson et al. (1993); Tarodi et al. (1994); Young et al. (1999)</td>
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<td>LMP-1</td>
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<td>Interacts with TRAFs and TRADD and upregulates NFκB</td>
<td>Low serum</td>
<td>–</td>
<td>Devergne et al. (1990); Kawanishi (1997); Mosialos et al. (1998)</td>
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<td></td>
<td></td>
<td>Induces the expression of Bcl-2 and A20</td>
<td>Low serum</td>
<td>–</td>
<td>Henderson et al. (1991); Laherty et al. (1992); Young et al. (1999)</td>
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<td>Vockerodt et al. (2001)</td>
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<td>Equine herpesvirus</td>
<td>BALF1</td>
<td>Bcl-2-related protein</td>
<td>IFN-γ</td>
<td>–</td>
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<td>E8</td>
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<td>Signalling pathway – DED-mediated interaction</td>
<td>TNF-α and anti-Fas</td>
<td>–</td>
<td>Bertin et al. (1997)</td>
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<td></td>
<td></td>
<td>Contains death effector domains and interacts with caspase-8</td>
<td>TNF receptor 1 and Fas</td>
<td>–</td>
<td>Hu et al. (1997)</td>
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<td>HSV</td>
<td>γ$_1$34.5 gene</td>
<td>IFN-mediated pathway. Decreases eIF-2α phosphorylation by PKR</td>
<td>–</td>
<td>+</td>
<td>Cassady et al. (1998); Chou &amp; Roizman (1992); Randazzo et al. (1997)</td>
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<td></td>
<td></td>
<td>Ser/Thr kinase that prevents virus-induced apoptosis</td>
<td>UV irradiation and anti-Fas</td>
<td>+</td>
<td>Jerome et al. (1999)</td>
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<td>U$_3$</td>
<td>Cooperates with U$_3$</td>
<td>UV irradiation and anti-Fas</td>
<td>+</td>
<td>Jerome et al. (1999)</td>
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<td>Virus</td>
<td>Anti-apoptosis gene(s) or protein(s)</td>
<td>Mechanism of action</td>
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<td>Human cytomegalovirus</td>
<td>IE1 and IE2</td>
<td>Transcriptional regulation. Controls CMV early transcription; blocks TNF apoptosis</td>
<td>TNF-α</td>
<td>+</td>
<td>Zhu et al. (1995)</td>
</tr>
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<td>vICA</td>
<td></td>
<td>Prevents caspase-8 activation</td>
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<td>Kaposi’s sarcoma herpesvirus</td>
<td>K13</td>
<td>Signalling–viral FLICE-inhibitory protein (vFLIP)</td>
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<td>–</td>
<td>Skaletskaya et al. (2001); Sturzl et al. (1999)</td>
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<td>Marek’s disease virus</td>
<td>MEQ</td>
<td>Transcriptional regulation – bZIP leucine zipper transexual factor</td>
<td>TNF-α, C2-ceramide, UV irradiation and low serum</td>
<td>–</td>
<td>Liu et al. (1998)</td>
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<tr>
<td>SV40</td>
<td>Large T</td>
<td>Mechanism independent of p53 inactivation</td>
<td>Low serum</td>
<td>–</td>
<td>Conzen et al. (1997); Fromm et al. (1994); Tsai et al. (2000)</td>
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<td>CPV</td>
<td>CrmA</td>
<td>Inhibition of caspases-1, -4, -5 and -11</td>
<td>TNF-α and anti-Fas</td>
<td>–</td>
<td>Dbaibo &amp; Hannun (1998); Tewari &amp; Dixit (1995); Zhou et al. (1997)</td>
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<td>MOCV</td>
<td>GPx</td>
<td>Oxidative stress – scavenges reactive oxides</td>
<td>UV irradiation and H₂O₂</td>
<td>–</td>
<td>Moss et al. (2000); Shisler et al. (1998)</td>
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<td>(MC066L)</td>
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<td>MYXV</td>
<td>M-T2</td>
<td>TNF receptor-related protein – inhibits TNF-α apoptosis (extracellular) and blocks T cell apoptosis (intracellular)</td>
<td>TNF-α</td>
<td>+</td>
<td>Macen et al. (1996); Schreiber et al. (1997); Xu et al. (2000)</td>
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Table 2 (cont.)

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<th>Virus</th>
<th>Anti-apoptosis gene(s) or protein(s)</th>
<th>Mechanism of action</th>
<th>Apoptotic stimulus</th>
<th>Recombinant virus lacking gene or active protein*</th>
<th>Reference</th>
</tr>
</thead>
<tbody>
<tr>
<td>M-T4</td>
<td>Retention in the endoplasmic reticulum, inhibits T cell apoptosis</td>
<td>Seen with infection of deletion mutant</td>
<td>+</td>
<td>Barry et al. (1997); Hnatiuk et al. (1999)</td>
<td></td>
</tr>
<tr>
<td>M11L</td>
<td>Prevents the mitochondria from undergoing a permeability transition. Inhibits apoptotic response of macrophages and monocytes</td>
<td>Staurosporine</td>
<td>+</td>
<td>Everett et al. (2000); Macen et al. (1996)</td>
<td></td>
</tr>
<tr>
<td>Serp2</td>
<td>Weak inhibitor – cannot protect CPV-infected cells from apoptosis</td>
<td>Interleukin-1β-converting enzyme and granzyme B</td>
<td>+</td>
<td>Messud-Petit et al. (1998); Turner et al. (1999)</td>
<td></td>
</tr>
<tr>
<td>VV</td>
<td>Modulates the mitochondrial permeability transition pore to staurosporine</td>
<td>Anti-Fas or staurosporine</td>
<td>–</td>
<td>Waslenko et al. (2001)</td>
<td></td>
</tr>
<tr>
<td>SPI-2</td>
<td>Similar to CrmA</td>
<td>Anti-Fas and TNF-α</td>
<td>+</td>
<td>Dobbelslein &amp; Shenk (1996)</td>
<td></td>
</tr>
<tr>
<td>(B13R) Ectromelia virus SPI-2</td>
<td>Inhibits interleukin-1β-converting enzyme</td>
<td>Anti-Fas and TNF-α</td>
<td>+</td>
<td>Kettle et al. (1997)</td>
<td></td>
</tr>
<tr>
<td>(B13R) Retroviridae Retrovirus SPI-2</td>
<td>Inhibited caspases-1 and -8 (but not -3, -6 or granzyme B)</td>
<td>TNF-α</td>
<td>–</td>
<td>Turner et al. (2000)</td>
<td></td>
</tr>
<tr>
<td>GPx</td>
<td>Oxidative stress – detoxifies peroxides and free radicals</td>
<td>–</td>
<td>–</td>
<td>Zhao et al. (2000)</td>
<td></td>
</tr>
<tr>
<td>Tat</td>
<td>Phosphatidylinositol 3 and Akt/PKB kinase pathway</td>
<td>Low serum</td>
<td>–</td>
<td>Borgatti et al. (1997); Gibellini et al. (1995)</td>
<td></td>
</tr>
<tr>
<td>Vpr</td>
<td>Normally pro-apoptotic Vpr interrupts TCR-mediated apoptosis. Regulation of NFκB</td>
<td>Sorbitol</td>
<td>–</td>
<td>Ayyavoo et al. (1997); Fukumori et al. (1998)</td>
<td></td>
</tr>
<tr>
<td>p17/p24</td>
<td>Production of antagonistic epitopes that inhibit the normal lysis by cytotoxic T lymphocytes</td>
<td>Cytotoxic T cell activity</td>
<td>+</td>
<td>Klenerman et al. (1994)</td>
<td></td>
</tr>
</tbody>
</table>

*A virus that has had a recombinant constructed that lacks the ability to produce an active form of this protein is indicated by ‘−’, whereas a recombinant virus that lacks the ability to produce an active form of this protein is indicated by ‘+’.
was shown in a recent study involving an adenovirus vector that proliferated in human cancer cell lines (Mi et al., 2001). The vector sensitized the infected cells to recombinant tumour necrosis factor (TNF)-z-mediated PCD by the expression of dominant-negative I-xB. The results showed that, while apoptosis during viral DNA replication was detrimental to the proliferation of the virus, apoptosis after virion assembly enabled its dissemination. Electron microscopy showed the occurrence of virus within the apoptotic bodies or associated with them. This later induction of apoptosis also allowed for the spread of infection and subsequent regression in subcutaneous tumours or liver metastases (Mi et al., 2001). Viruses are clearly sophisticated in their strategies for dissemination. In addition to the modulation of the apoptotic pathways, they may also bud from present and future contact points with other cells, further facilitating the spread of infection (Johnson & Huber, 2002).

It is believed that viruses that manipulate the apoptotic pathways have captured and modified the cellular machinery that regulates the host cell death pathways (McFadden et al., 1998). The selective pressure for these types of survival promoting alterations would be immense and the advantage would be immediate.

It is not uncommon for a single virus to encode proteins that function to both promote and inhibit apoptotic death. Human immunodeficiency virus type 1 (HIV-1) is such a virus. Its arsenal is formidable in its duality and in its potentiality. HIV Nef is able to induce apoptosis by way of the signalling pathway; it mediates an increased expression of TNF on the cell surface while also downregulating MHC class I and CD4 expression (Lama & Ware, 2000). HIV Tat is also able to induce death by the extrinsically mediated pathway through increased expression of Fas (CD95) (Li-Weber et al., 2000). However, other mechanisms by which Tat is able to induce apoptosis have been published (Bonavia et al., 2001; New et al., 1998; Park et al., 2001). In addition, the effect of Tat in vivo is questionable to some degree given the incidence of abnormally high concentrations used experimentally. HIV also utilizes gp120/gp41 and Vpr for its pro-apoptotic imperatives. The gp120/gp41 is a complex derived from the envelope region of the retrovirus genome. The resulting complex cross-links to CD4 lymphocytes and induces apoptosis (Laurent-Crawford et al., 1993, 1995; Sylwestler et al., 1997). The mechanism used by Vpr remains somewhat enigmatic. It is known that this protein can induce cell cycle arrest in the G2 phase and that this induction is related to the promotion of apoptosis (Chang et al., 2000; Conti et al., 2000; Patel et al., 2000; Stewart et al., 1997; Zhu et al., 2001). Opposing this death effect are G'2X glutathione peroxidase, a protein that acts to reduce oxidative stress, and variants of the gag core protein p17–p24 that produce an antagonistic epitope effect and thereby inhibit apoptotic death via CD8+ leucocytes (Klenerman et al., 1994; Zhao et al., 2000).

Indeed, data have been shown that the normally apoptotic proteins Tat and Vpr are able to evade the death sequence by elevating internal Bcl-2 levels and by interrupting the T cell receptor (TCR)-mediated apoptotic process, respectively (Ayyavoo et al., 1997; Fukumori et al., 1998; McCloskey et al., 1997; Zauli et al., 1993). Moreover, the effect of Tat when expressed exogenously has been demonstrated to inhibit the TNF-related apoptosis-inducing ligand (TRAIL)-mediated death pathway (Gibellini et al., 2001). It is hypothesized that the dual internal function of Tat and Vpr may act to regulate the stages of virus infection. These factors may indeed play an important role in the chronology of virus replication and the subsequent apoptotic cell demise during infection (Conti et al., 2000). Overall, HIV serves as a striking example for the depth of complexity often seen within these ‘simple’ organisms. Clearly, the effective exploitation of the apoptotic pathway, above all other cell processes, is a determining factor in virus survival.

Tables 1 and 2 incorporate some of the known pro-apoptotic and anti-apoptotic factors produced by viruses. Here, the virus mechanisms for manipulating apoptosis are discussed.

### Signalling mediators (TNF and Fas)

Initiation of the Fas and TNF apoptosis pathways occurs by the binding of receptor and ligand on the cell surface, resulting in the stimulation of a distinct cytoplasmic apoptosis pathway as shown in Fig. 1. Often, this pathway is referred to as the ‘extrinsically mediated pathway’, apparently due to its external activation. From the point of initiation, viruses influence this pathway. Adenoviruses in particular have a direct influence at an early stage, with proteins E3-10.4K and E3-14.5K, which reduce the presentation of Fas molecules on the surface of the cell. This reduction of the apoptotic receptor results in a resistance to Fas-mediated cell death. The proteins 10.4K/14.5K also confer a protective effect against TNF-mediated apoptosis. However, in this case a loss of TNF surface expression is not responsible, indicating multiple cell death protecting qualities for this complex (Dimitrov et al., 1997; Shisler et al., 1997). Epstein–Barr virus (EBV) produces an anti-apoptotic protein named LMP-1 (latent membrane protein-1). This protein with its six membrane-spanning domains accumulates in the host cell plasma membrane. LMP-1 appears to be a constitutively activated TNF receptor, which is ligand-independent (Gires et al., 1997). It has been shown that LMP-1 interacts with TNF-associated factors (TRAFs). TRAFs 1, 2 and 3 all bind to a site in the C-terminal domain between amino acids 199 and 214, a region known to be of significance for B cell growth transformation (Devergne et al., 1996). In 1997, Izumi & Kieff (1997) also reported another site, TES2 (transformation effector site 2), which interacts with a TNF receptor-associated death domain protein (TRADD). Indeed, it utilizes TRADD in a signalling pathway in a manner similar to TNF receptor 1. The TES2 interaction with TRADD mediates...
a high-level activation of NFκB, in contrast to the association of TES1 with TRAFs, which only accounts for low-level NFκB activation (Devergne et al., 1996; Eliopoulos et al., 1999; Gires et al., 1997; Izumi & Kieff, 1997; Kusano & Raab-Traub, 2001; Mosialos et al., 1995; Roberts & Cooper, 1998).

The myxoma virus (MYXV) protein M-T2 is a virus mimic protein of the TNF receptor and is a potent inhibitor of extracellular TNF-mediated apoptosis. It is released in either a monomeric or a dimeric form and binds TNF, thus negating TNF receptor signal transduction. The protein also has an intracellular ability to suppress cell death in CD4+ T lymphocytes. This function is distinct to that of binding extracellular TNF and appears to be mediated by the first two cysteine-rich domains of the M-T2 protein (Barry & McFadden, 1998; McFadden & Barry, 1998; Schreiber et al., 1997; Xu et al., 2000). Cowpox virus (CPV) also has TNF receptor family-related proteins, the cytokine response-modifying (Crm) proteins CrmB, CrmC and CrmD. M-T2 has similarity to both CrmB and CrmD (Cunnion, 1999). Until recently, it was unknown whether the C-terminal regions of the Crm proteins had TNF ligand-binding domains. It has now been shown that the vaccinia virus (VV) protein related to CrmC, A53R, is
capable of producing a TNF-binding protein. This protein is soluble and able to bind TNF with high affinity, thus preventing the engagement of cell TNF receptors (Alcamí et al., 1999).

IFN pathway mediators

A host response to virus infection includes the induction of apoptosis of the infected cell through the action of leucocytes. IFNs participate in this process. These proteins exhibit signalling potential and have the ability to modulate immune activity and cell proliferation (Balachandran et al., 2000). However, it is the ability of these proteins to interfere with the apoptotic pathway that is of particular interest here. In this regard, IFN plays an important part of a hosts’ response to infection and it achieves this by effecting important cellular genes, such as the dsRNA-dependent serine/threonine protein kinase (PKR), resulting in the sensitization of the cell death machinery. Any stage in virus infection appears susceptible to its inhibitory capacity (Stark et al., 1998). In effect, IFNs prime a cell to destruction through the FADD (Fas-associated death domain)-containing protein-dependent apoptotic pathway. Furthermore, IFN is able to prevent the replication of some viruses that circumvent the activation of this pathway, hence the cell avoids the death program altogether (Balachandran et al., 2000; Ezelle et al., 2001). The significance of IFN to the virus is clearly apparent. Yet, it is surprising that, given its central importance, we do not see more reports of manipulation of the IFN-mediated pathway by viruses – though perhaps this remains a matter of time.

Nevertheless, both VV and herpes simplex virus (HSV) work to affect this pathway through PKR. This enzyme, along with RNase L, is a key player in the IFN-mediated apoptotic pathway. Both of these enzymes are activated by the dsRNA that is often produced during the course of a virus infection (Barry & McFadden, 1998; Der et al., 1997; Diaz-Guerra et al., 1997; Kibler et al., 1997). It has been shown that PKR and IFN sensitize cells through the FADD/caspase-8 pathway (Balachandran et al., 2000). It is interesting that RNase L, though acting in a similar manner, functions separately from PKR in response to apoptotic stimuli. Similarly, RNase L shows an ameliorated response to dsRNA. It was, however, still able to induce death effectively in cells from PKR-deficient mice (Diaz-Guerra et al., 1997).

Recently, Ezelle et al. (2001) provided further evidence of virus interaction with the IFN pathway and they hypothesized that viruses target not only the IFN inducible genes but also effectors along the IFN pathway. This suggests that viruses could avoid apoptotic cell death while allowing the expression of primary IFN targets such as the otherwise integral antiviral protein PKR.

Bcl-2-related proteins

The Bcl-2 protein family has at least 15 members that have been identified in mammalian cells, with still more evident in viruses (Adams & Cory, 1998; Chao & Korsmeyer, 1998; Cory, 1994, 1995; Strasser et al., 1997). The members of this large family all contain at least one of the four conserved Bcl-2 homology domains, titled BH1 to BH4.

Bcl-2 itself is an integral membrane protein located mainly on the outer membrane of the mitochondria (Yang et al., 1997). It can prevent apoptosis mediated by some caspases, but not all (Sutton et al., 1997). The Bcl-2 protein family is able to modulate the cytochrome c/Apaf-1/caspase-9 pathway by essentially regulating the liberation of cytochrome c (Kluck et al., 1997; Yang et al., 1997). The anti-apoptotic members such as Bcl-2 and Bcl-XL act to mitigate its release, while Bad, Bax, Bid and Bim move from other cellular organelles to the mitochondria in response to apoptotic stimuli where they encourage the release of the molecule (Gross et al., 1999).

Bcl-2-related proteins have a number of virus mimics. The E1B-19K protein of adenovirus is such a mimic. It has been shown to associate with and inhibit apoptosis induced by the pro-apoptotic Bcl-2 protein family members, such as Bak, Bik and Bax (Granville et al., 1998; Rao et al., 1997). The adenoviral protein also interacts with the nuclear lamin, an interaction that has been shown to be necessary for both the localization of E1B-19K and the inhibition of apoptosis (Barry & McFadden, 1998; Rao et al., 1997). This protein inhibits a specific form of Bax and, as a result, blocks the TNF-α-mediated death-signalling pathway. The caspase cascade is stopped upstream of caspase-9 but downstream of caspase-8 (Perez & White, 2000).

BHRF1, a 17 kDa EBV protein, also bears similarity to Bcl-2, in both its structure and its function (Fanidi et al., 1998; Khanim et al., 1997; Niedobitek et al., 1991). BHRF1 has been shown specifically to bear 38% homology to the C-terminal portion of Bcl-2 and to bear the same ultra-structural localization while also being able to inhibit c-Myc-induced apoptosis (Fanidi et al., 1998; Hickish et al., 1994).

Recently, another related protein was discovered from the same virus. This virus survival gene, BALF1, encodes a protein that suppresses apoptosis and is associated with Bax and Bak (Marshall et al., 1999; Young et al., 1999). Structural analysis of BALF1 reveals several regions that recommend it as a Bcl-2 protein family member. Significantly, this includes sequence similarity in the Bcl-2 homologous domains BH1 to BH4, a region known to be important for function. It is intriguing that BALF1 shows a closer relationship to Bcl-xL and Bcl-2 than between the known EBV-encoded Bcl-2-related protein BHRF1 (Marshall et al., 1999).

Caspase suppressors

There are few identified virus caspase inhibitors, amongst those discovered are CPV CrmA, baculovirus IAP (inhibitor of apoptosis protein) and p35 and African swine fever virus (ASFV) product A224L/4CL. CrmA is a potent caspase-1/caspase-8 inhibitor (Muzio et al., 1996; Zhou et al., 1997).
CrmA does have some inhibition qualities for other caspases but the effect is substantially diminished (Kamada et al., 1997; Zhou et al., 1997). On the other hand, baculovirus protein p35 is a wide-ranging caspase inhibitor, inhibiting mammalian caspases 1–4 and 7 (Barry & McFadden, 1998; Miller, 1997). Even though the caspase inhibition qualities differ between these virus agents, they share the ability to inhibit TRAIL-induced apoptosis (Suliman et al., 2001).

The other anti-apoptotic baculovirus protein is IAP. It has become the archetypal member for a protein family in a way similar to Bcl-2. Its members, though not all appearing to be involved in apoptosis, show between one and three baculovirus repeats (Ekert et al., 1999). Mammalian proteins related to this viral protein that have been shown to have an inhibitory effect on apoptosis include XIAP, c-IAP1, c-IAP2 and survivin (Deveraux & Reed, 1999). The ASFV gene A224L produces a viral IAP-related protein (Chacon et al., 1995). This protein has been shown to interact with and suppress the activity of caspase-3 (Nogal et al., 2001).

**Cell cycle manipulators**

Distinct from most other categories, the cell cycle regulators are comprised almost equally of apoptotic inhibitors and inducers. The large T antigen expressed by simian virus 40 (SV40) is a good example, having in itself, both qualities. The antigen binds the retinoblastoma tumour suppressor protein (pRb)/p107/p130, in addition to p53 (Barry & McFadden, 1998). This antigen can suppress caspase-1-induced apoptosis in a p53-dependent fashion (Jung & Yuan, 1997). However, apoptosis is also promoted by the large T antigen binding to pRb (Kolzau et al., 1999). This binding of pRb allows an increase in the transcriptional activity of E2F through its liberation, which triggers expression of p73 (Iglesias et al., 1998). Furthermore, SV40 has also a small tumour antigen, which inhibits the apoptosis-inducing qualities of the large T antigen, although the mechanism by which it achieves this still remains unknown (Kolzau et al., 1999).

The pX protein of hepatitis B virus (HBV) is also able to bind p53 and regulate the cell cycle. The controlled expression of this protein acts to modulate p53-dependent apoptosis (Chirillo et al., 1997; Elmore et al., 1997; Kim et al., 1998; Lin et al., 1997). An increase in the production of pX induces apoptosis, while a decline inhibits apoptosis (Chirillo et al., 1997; Elmore et al., 1997; Kim et al., 1998; Su & Schneider, 1997). The pX protein associates with p53 in the cytoplasm and minimizes its movement to the nucleus, where it acts to transactivate p53-responsive genes. Adding complexity to the issue is that, in the absence of p53, pX acts to promote cell proliferation rather than cell death; this appears to be the result of its alternate function as an independent transcriptional coactivator (Barnabas et al., 1997; Barry & McFadden, 1998; Chirillo et al., 1997; Elmore et al., 1997; Kim et al., 1998; Lin et al., 1997; Su & Schneider, 1997; Takada et al., 1997).

**Oxidative stress regulators**

Oxidative stress is a well-established feature of apoptosis. Certainly, the production of reactive oxygen intermediates and the accumulation of oxidized cellular compounds play a role in cell death mediation (Buttke & Sandstrom, 1994; McGowan et al., 1996). In the cell, GPx manages the deleterious effects of these stressing agents. Yet, it has been shown that molluscum contagiosum virus (MOCV) is also able to encode a similar protein to this cellular selenoprotein (Shisler et al., 1998). This protein apparently confers a survival advantage by inhibiting apoptosis. Transfection of the GPx gene, MC066L, into keratinocytes or HeLa cells inhibited apoptosis instigated by hydrogen peroxide or UV irradiation but not by TNF or Fas (Barry & McFadden, 1998; Shisler et al., 1998). More recently, however, it has been shown that HIV-1 produces the same active selenoprotein (Zhao et al., 2000). Zhang et al. (1999) found through sequence database searching combined with structurally guided comparative sequence analysis that GPx molecules may be produced in a number of RNA viruses, including, HCV, coxsackievirus B3, HIV-2 and measles virus (Zhang et al., 1999).

**Protein kinases**

U₅₃, a viral gene encoding an anti-apoptosis protein, is a product of HSV that functions as a serine/threonine kinase to block virus-induced apoptosis. This enzyme phosphorylates serine/threonine within a specific arginine-rich consensus sequence (Leopardi et al., 1997). The details of the phosphorylating action of U₅₃ remain undetermined. It is known that U₅₃ cooperates with U₅₅ to suppress apoptosis. These proteins function to strongly inhibit apoptosis induced by the Fas receptor or UV irradiation. U₅₅ appears to be more critical for the inhibition of Fas-mediated apoptosis than U₅₃ (Jerome et al., 1999).

**Transcriptional modifiers**

It is to be expected that if viruses were to possess the machinery necessary to effect matters of cell survival and cell death that they should have the means to regulate transcription. Papillomaviruses achieve this by synthesis of a protein, E2, which represses the transcription of the integrated gene for E6, and by another p53-reliant scheme, which results in G₁ arrest and apoptosis (Desaintes et al., 1997). Marek’s disease virus apparently works by controlling the transcription of apoptosis-related genes, for example, by the induction of bcl-2 and the repression of Bax (Barry & McFadden, 1998; Liu et al., 1998).

**Endoplasmic reticulum-targeting factor**

M-T4, the fourth MYXV gene, is a critical virulence factor for myxomatosis in infected rabbits. It has an unusual attribute
in that it is specifically targeted back to the endoplasmic reticulum. The presence of a C-terminal RDEL sequence is thought to be a causative region for this transportation to the endoplasmic reticulum (Barry et al., 1997). However, this region may not be solely responsible for protein movement (Hnatiuk et al., 1999).

Recently, rubella virus was shown to induce apoptosis via its capsid protein, which was also located in the endoplasmic reticulum. It has also been demonstrated that the apoptotic effect of the protein is not linked to the intra-organelle calcium capacity (Duncan et al., 2000).

Conclusion
Virus regulation of the apoptotic cell death program is a highly developed process. Viruses have shown repeatedly throughout history their prowess at manipulating this integral pathway; a fact demonstrated the world over by virus epidemics such as AIDS, polio and smallpox. A virus may with relative ease enter a cell and inhibit apoptosis in a manoeuvre that allows it sufficient time to proliferate, disperse from the plasma membrane and to infect other cells. Conversely, though just as readily, a virus may infect a cell, induce it to die and then spread to neighbouring cells through the phagocytosis of the resulting apoptotic bodies, in the process minimizing an immune response. Viruses are able at their task. Collectively they have an arsenal of proteins for both defence and offence. In many ways, the cell in all its complexity may be viewed as a marionette to the virus. Although the virus may only have a genome of several 1000 bp, it still has the capacity to pull the most crucial of ‘strings’ in a host that dwarfs it in terms of size and genetic material.

The selective pressure for the incorporation of survival promoting genetic alterations in a virus, such as the capture of Bcl-2-related genes would be enormous. However, the number of ways in which viruses manipulate the apoptotic mechanism must illustrate not only the selective advantage of such insertions but also the susceptibility of this crucial process to manipulation and corruption.

Although viruses can cause tremendous harm, the recent advances in molecular biology herald an era where the genes from these parasites may be used to generate a panoply of potential therapeutic benefits. In such a case, proliferative diseases such as cancer may be aided by apoptotic genes derived from viruses and targeted to the multiplying cells, while degenerative diseases such as Alzheimer’s, Parkinson’s and Huntington’s diseases may similarly be treated with anti-apoptotic viral genes. Ideologically, the process seems quite simple but difficulties are apparent when details such as gene delivery, expression levels and the time of gene expression are considered (Kay et al., 2001; Verma & Somia, 1997). The genetic structure of a virus recommends itself for gene therapy given its inherent simplicity. The fact that viruses are naturally infective and naturally able to alter a cells course between life and death make them a good starting point. However, as more knowledge is gained about cellular systems, it is likely that we may borrow more heavily from the existing cellular repertoire or simply engineer more efficient vectors in the name of expediency. One argument in favour of virally derived gene therapies is that these systems have presumably undergone considerable recombination and selection throughout their history. Consequently, they represent an amalgam of elements effective in enhancing the infection of viral (and potentially other) genetic elements.

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References


Review: Virus-induced apoptosis


