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REVIEW ARTICLE

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

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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, phosphatidlyserine 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

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Table 1. Viruses that produce apoptosis-inducing proteins

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

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

Table 1 (*cont.*)

Virus	Pro-apoptosis gene(s) or protein(s)	Mechanism of action	Apoptosis induction seen in	Recombinant virus lacking gene or active protein*	Reference
Papovaviridae					
SV40	Large T	Blocks function of pRb (retinoblastoma) and p53 Apoptosis induction dependent on pRb binding	Mouse lens	+	Fromm *et al.* (1994); Kolzau *et al.* (1999); McCarthy *et al.* (1994)
Retroviridae					
Human immunodeficiency virus	Tat	Increased activation of cyclin-dependent kinases	T cell line and PBMCs	−	Li *et al.* (1995)
		Decreased expression of manganese-dependent superoxide dismutase	Jurkat and HeLa	+	Westendorp *et al.* (1995)
		Amelioration of Fas-dependent signal transduction via a TNF-α mechanism	Human and rat neurons	−	Kolesnitchenko *et al.* (1997); New *et al.* (1998)
		Modulation of cAMP and CREB levels	PC12	−	Zauli *et al.* (2001)
		Upregulation of caspase 8	Jurkat	+	Bartz & Emerman (1999)
		Increase of Fas (CD95) by a process involving NFκB	Not tested	−	Li-Weber *et al.* (2000)
	gp120/gp41	Syncytium formation and CD4 cross-linking	MOLT4-T4 and H9	−	Laurent-Crawford *et al.* (1993, 1995); Maldarelli *et al.* (1995); Sylwester *et al.* (1997)
	Vpr	Caspase-8-mediated pathway and modulated by the Bcl-2 family	SupT1 and HeLa	+	Chang *et al.* (2000); Conti *et al.* (2000); Patel *et al.* (2000); Stewart *et al.* (1997)
	Nef	Increased expression of TNF on cell surface	II-23.D7	+	Lama & Ware (2000)
Togaviridae					
Semliki Forest virus	Not known	Induces p53-independent apoptosis from the non-structural part of the genome	H358a and neurons	+	Allsopp *et al.* (1998); Murphy *et al.* (2000)
Sindbis virus	E1 and E2	Virus entry – initiates sphingomyelin degradation and ceramide release	AT3	−	Jan *et al.* (2000); Joe *et al.* (1998)
Rubella virus	Capsid	Endoplasmic reticulum-localized factor	Vero and RK13	−	Duncan *et al.* (2000); Law *et al.* (2001); Pugachev & Frey (1998)

* 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

Virus	Anti-apoptosis gene(s) or protein(s)	Mechanism of action	Apoptotic stimulus	Recombinant virus lacking gene or active protein*	Reference
Adenoviridae					
Adenovirus	E1B-19K	Bcl-2 type – Inhibits E1A, Fas, TNF-induced apoptosis specific form of Bax – caspase activation stopped before caspase-9 but after caspase-8	TNF-α	+	Farrow *et al.* (1995); Marcellus *et al.* (1996a); Perez & White (2000)
		Binds to nuclear lamins	E1A-induced p53-dependent	–	Rao *et al.* (1997)
		Bcl-2 family	E1A-induced p53-dependent	–	Debbas & White (1993)
	E1B-55K	Binds to p53 and functionally inactivates it	E1A-induced p53-dependent	–	Teodoro & Branton (1997b); White *et al.* (1992); Yew *et al.* (1994)
	E3-6.7	Complexes with 10.4 and 14.5 resulting in downmodulation of TRAIL receptor 1 and 2	TRAIL and FasL	+	Benedict *et al.* (2001)
	E3-10.4	Inhibits E1A or TNF-induced apoptosis	TNF-α	+	Gooding *et al.* (1988, 1991)
		Inhibits TNF-induced release of arachidonic acid	TNF-α	+	Krajcsi *et al.* (1996)
		Inhibits TNF-induced transfer of PLA2 to cell membrane	TNF-α	+	Dimitrov *et al.* (1997)
		Decreased presentation of Fas on the cell surface	Anti-Fas	+	Shisler *et al.* (1997)
	E3-14.5	Inhibits E1A or TNF-induced apoptosis	TNF-α	+	Gooding *et al.* (1991)
		Inhibits TNF-induced release of arachidonic acid	TNF-α	+	Krajcsi *et al.* (1996)
		Inhibits TNF-induced transfer of PLA2 to cell membrane	TNF-α	+	Dimitrov (1997)
		Decreased presentation of Fas on the cell surface	Anti-Fas	+	Shisler *et al.* (1997)
	E3-14.7K	Inhibits E1A or TNF-induced apoptosis	TNF-α	+	Gooding *et al.* (1988)
		Caspase pathway – caspase-2 and -8	TNF-α	–	Li *et al.* (1998)
		Inhibits TNF-induced release of arachidonic acid	TNF-α	+	Krajcsi *et al.* (1996)
African swine fever-like viruses					
ASFV	LMW5-HL/A179L	Bcl-2-related protein	Protein kinase (p68)	–	Afonso *et al.* (1996); Brun *et al.* (1996, 1998); Neilan *et al.* (1993); Revilla *et al.* (1997)
	A224L	IAP-related protein	TNF-α and cycloheximide or staurosporine	+	Chacon *et al.* (1995); Nogal *et al.* (2001)

Table 2 (*cont.*)

Virus	Anti-apoptosis gene(s) or protein(s)	Mechanism of action	Apoptotic stimulus	Recombinant virus lacking gene or active protein*	Reference
Baculoviridae					
Baculovirus	P35	Caspase inhibitors 1, 3, 6–8 and 10	Staurosporine and extracellular potassium	+	Bertin *et al.* (1996); Clem *et al.* (1991); Viswanath *et al.* (2000); Zhou *et al.* (1998)
		Inhibits oxidative stress-induced apoptosis	H_2O_2	+	Sah *et al.* (1999)
	IAP	Caspase inhibition – caspase-3, -6 and -7	Actinomycin D	+	Crook *et al.* (1993); Deveraux *et al.* (1997)
Herpesviridae					
Bovine herpesvirus-4	BORFE2	Inhibits Fas and TNF-induced apoptosis. Contains death effector domains and interacts with caspase-8	Fas and TNF receptor 1	–	Wang *et al.* (1997)
EBV	BHRF1	Bcl-2-related protein	Low serum, ionomycin, cisplatin and etoposide	–	Henderson *et al.* (1993); Tarodi *et al.* (1994); Young *et al.* (1999)
	LMP-1	Interacts with TRAFs and TRADD and upregulates NFκB	Low serum	–	Devergne *et al.* (1996); Kawanishi (1997); Mosialos *et al.* (1995)
		Induces the expression of Bcl-2 and A20	Low serum	–	Henderson *et al.* (1991); Laherty *et al.* (1992); Young *et al.* (1999)
		Interleukin-10 production upregulated	–		Vockerodt *et al.* (2001)
	BALF1	Bcl-2-related protein	IFN-γ	–	Marshall *et al.* (1999)
Equine herpesvirus	E8	Signalling pathway – DED-mediated interaction	TNF-α and anti-Fas	–	Bertin *et al.* (1997)
		Contains death effector domains and interacts with caspase-8	TNF receptor 1 and Fas	–	Hu *et al.* (1997)
HSV	$\gamma_1$34.5 gene	IFN-mediated pathway. Decreases eIF-2α phosphorylation by PKR	–	+	Cassady *et al.* (1998); Chou & Roizman (1992); Randazzo *et al.* (1997)
	U_S3	Ser/Thr kinase that prevents virus-induced apoptosis	UV irradiation and anti-Fas	+	Jerome *et al.* (1999)
	U_S5	Cooperates with U_S3	UV irradiation and anti-Fas	+	Jerome *et al.* (1999)

Table 2 (*cont.*)

Virus	Anti-apoptosis gene(s) or protein(s)	Mechanism of action	Apoptotic stimulus	Recombinant virus lacking gene or active protein*	Reference
Human cytomegalovirus	IE1 and IE2	Transcriptional regulation. Controls CMV early transcription; blocks TNF apoptosis	TNF-α	+	Zhu *et al.* (1995)
	vICA	Prevents caspase-8 activation	Anti-Fas, TNF-α and TRAIL	–	Skaletskaya *et al.* (2001)
Kaposi's sarcoma herpesvirus	K13	Signalling–viral FLICE-inhibitory protein (vFLIP)	Low serum	–	Low *et al.* (2001); Sturzl *et al.* (1999)
	(ORF17)			–	Djerbi *et al.* (1999)
Marek's disease virus	MEQ	Transcriptional regulation – bZIP leucine zipper transcriptional factor	TNF-α, C2-ceramide, UV irradiation and low serum	–	Liu *et al.* (1998)
Papovaviridae					
Human papillomavirus	E6	Cell cycle – Binds and degrades p53	E7	–	Pan & Griep (1995)
		Inhibits p53-independent and -dependent apoptosis		–	Thomas & Banks (1998, 1999)
		Degrades Bak			
SV40	Large T	Mechanism independent of p53 inactivation	Low serum		Conzen *et al.* (1997); Fromm *et al.* (1994); Tsai *et al.* (2000)
		Blocks caspase-1-induced apoptosis	Interleukin-1β-converting enzyme	–	Jung & Yuan (1997)
		Inhibition of p53-independent apoptosis via Rb family	Epidermal growth factor withdrawal	–	Slinskey *et al.* (1999)
Poxviridae					
CPV	CrmA	Inhibition of caspases-1, -4, -5 and -11	TNF-α and anti-Fas	–	Dbaibo & Hannun (1998); Tewari & Dixit (1995); Zhou *et al.* (1997)
MOCV	GPx	Oxidative stress – scavenges reactive oxides	UV irradiation and H_2O_2	–	Moss *et al.* (2000); Shisler *et al.* (1998)
	(MC066L)			–	
	MC159	Signalling pathway – DED-mediated interaction	TNF-α and anti-Fas	–	Bertin *et al.* (1997)
		Binds to procaspase-8 and FADD	Anti-Fas	–	Shisler & Moss (2001); Tsukumo & Yonehara (1999)
		Inhibits NF$\kappa\beta$ activation and dsRNA-dependent protein kinase-induced apoptosis	PKR	–	Gil *et al.* (2001)
MYXV	M-T2	TNF receptor-related protein – inhibits TNF-α apoptosis (extracellular) and blocks T cell apoptosis (intracellular)	TNF-α	+	Macen *et al.* (1996); Schreiber *et al.* (1997); Xu *et al.* (2000)

Table 2 (*cont.*)

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

* 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)-α-mediated PCD by the expression of dominant–negative I-κB. 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; Sylwester *et al.*, 1997). The mechanism used by Vpr remains somewhat enigmatic. It is known that this protein can induce cell cycle arrest in the G_2 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 GPx, 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

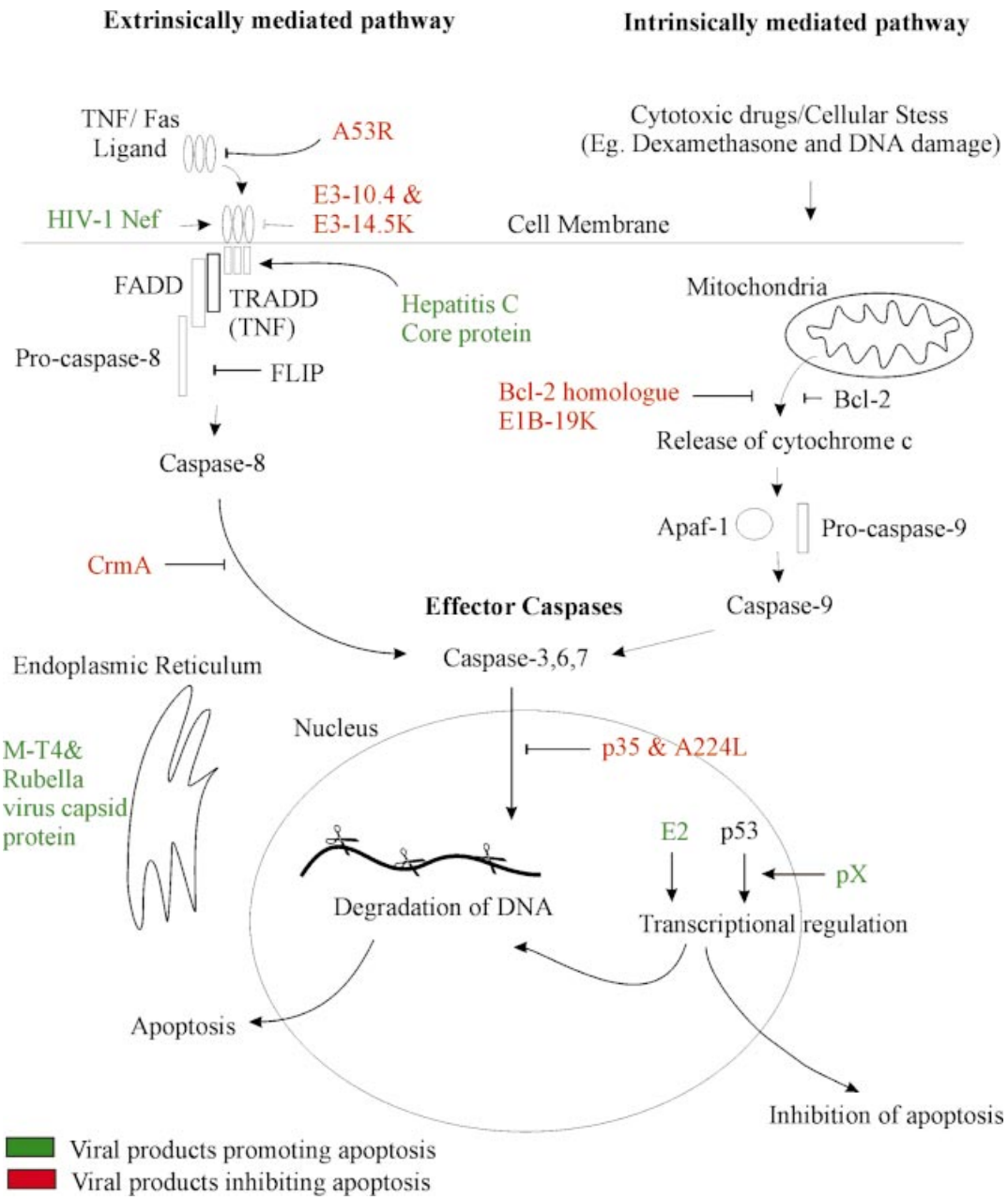

Fig. 1. The interaction of viral proteins with the apoptotic pathways of the host. Viral products promoting apoptosis are shown in green, whereas those inhibiting apoptosis are shown in red.

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-X_L 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

U_S3, 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 U_S3 remain undetermined. It is known that U_S3 cooperates with U_S5 to suppress apoptosis. These proteins function to strongly inhibit apoptosis induced by the Fas receptor or UV irradiation. U_S5 appears to be more critical for the inhibition of Fas-mediated apoptosis than U_S3 (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 G_1 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 susceptibly 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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